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United States Patent Office 2,794,786 Patented June 4, 1957

2,794,786

THIONIN DYE-ION EXCHANGE RESIN INDICATOR COMPOUNDS

Harry L. Segal and Leon L. Miller, Rochester, N. Y., assignors to Security Trust Company of Rochester, New York, as trustee for Harry L. Segal Medical Research Fund

No Drawing. Application May 27, 1955, Serial No. 511,786

10 Claims. (Cl. 260—2.2)

This application is a continuation-in-part of our earlier application Serial No. 146,384, filed February 25, 1950 (now abandoned), and our copending application Serial No. 212,698, filed February 24, 1951.

In our earlier applications there are disclosed novel non-toxic, resin ion exchange indicator compositions, administered orally, in which at least about 0.02 millimole of displaceable, identifiable indicator ion per dose (2 gm. dose) of resin exchanger is administered in unit dosage form for determining without intubation whether the pH of gastric juice in a stomach is above or below a predetermined pH value, thus providing a diagnostic indicator of particular value in clinical medicine.

In clinical diagnosis certain diseases such as pernicious anemia are associated with no free HCl in the stomach secretion. Carcinoma of the stomach is usually associated with very little or no free hydrochloric acid. Prior to this invention, it was necessary to subject the individual to the unpleasant procedure of introducing a tube into the stomach. The resin indicator compounds described here will obviate the necessity of the use of the intubation procedure which is not only unpleasant, but may also be difficult and even impossible under certain conditions.

The cure of gastric cancer depends on discovering the disease while it is still confined within the limits of the stomach. Since the disease is frequently silent, symptoms will not be of much aid even under the most enlightened circumstances. To apply routine mass fluoroscopy, G. I. X-ray series and conventional gastroscopy indiscriminately to all persons in the gastric cancer age group is impractical at present. Yet these diagnostic procedures are the only available means to discover the silent gastric cancer. The next best procedure might be the application of these available diagnostic techniques to a smaller group of individuals, who seem particularly liable to develop gastric cancer.

It is generally recognized that the incidence of gastric cancer is 3 to 4 times higher in people with achlorhydria than in the general population. The incidence may be ten times more frequent in the achlorhydrias with pernicious anemia. The incidence of achlorhydria in patients who have developed gastric cancer is 65% to 70%, as compared to that of only about 20% in the comparable age groups who are prone to develop carcinoma.

The ion exchange indicator compounds of this invention are prepared either by replacing the hydrogen cations of a carboxylic exchange resin with an equivalent number of an indicator cation or by adding an indicator anion to an anion exchange resin. The indicator cation or anion must be non-toxic in the dose employed, readily absorbed from the gastrointestinal tract after displacement from the compound and easily detectable in the body fluid chosen for its detection. The milliequivalents of the indicator ion displaced from a specific ion exchange indicator compound depends upon certain ion exchange properties such as the pH of the solution as well as the acid binding capacity of the ion exchange resin. The in vitro estimation of the number of milliequivalents of the indicator ion eluted from a gram of compound by a specific pH of gastric secretion and by the various cations of small intestinal juice, plus the knowledge of the percent of such eluted ions to appear in the urine or blood collected during or after a definitive interval makes it possible to predict the dose and the range of gastric acidity detectable by each compound. Thus, this invention allows the determination of gastric acidity by the displacement of the identifiable indicator ions from the indicator resin compounds of this invention by the hydrogen ions of the HCl in the stomach. The indicator ions used are detectable by simple or relatively simple procedures.

This tubeless method has the important clinical advantage in that the diagnostic agent has no therapeutic or pharmacological effect on the body. The distress and alterations in the composition of gastric juice which may be experienced with the intubation technique are completely eliminated. This tubeless procedure may even be more accurate than the intubation technique for determining the specific range of gastric acidity detectable by the specific indicator ion exchange resin. Any discrepancy noted between the results of the intubation and tubeless techniques done on different days can be usually explained on a physiological basis. Gastric acidity may fluctuate for various reasons. In some cases, as already indicated, the mere act of intubation produces a cessation of gastric secretion. Reichsmann, Engel and Segal have found modification of the gastric secretory response to histamine during varying behavioral states in an infant with a gastric fistula. There is also a group whose gastric acid secretion may vary from no free acid to a low acid range.

Since the commercial ion exchange resin is a well-recognized material established by previous clinical and medical experience to be insoluble, inert and non-toxic in rats, dogs and human beings and since the indicator ion selected is also non-toxic in the amount employed in the unit dosage, the indicator compound formed therefrom is found to retain the desirable non-toxic properties of the separate ingredients in its diagnostic application.

Suitable ion exchange resin indicator compounds in accordance wth this invention may be prepared from commercially available cation and anion exchange resins such as Amberlite IRC–50 (XE–96), a granular copolymer of methacrylic acid and divinyl benzene, Permutit H, a carboxylated phenol formaldehyde resin, and Permutit 70 or Amberlite IR–4B, a free synthetic phenolaldehyde resin and Amberlite IRA–400.

Thus, the ion exchange resin indicator compounds may be derived from either cation exchangers or anion exchangers depending upon the charge of the indicating ion which it is desired to incorporate into the ion exchange resin indicator compound.

The cation exchange resin such as a granular copolymer of methacrylic acid and divinyl benzene is conditioned, as described later, and then is treated with a solution containing a predetermined amount of the indicator ion in salt form. The resulting ion exchange resin indicator compound is removed from the solution, washed and dried, and thus prepared for use.

The displaceable indicator ions of the compounds of this invention may be selected from a group of dyes or other indicator ions which are known to be non-toxic and innocuous to humans in much larger doses than that used in these compounds. These cation or anion indicators are incorporated into the ion exchange resin indicator compound in quantitatively controlled amounts to provide the diagnostic indicator of this invention.

It has been discovered that the detection of the minimal quantity of gastric hydrochloric acid can be infallibly determined only if enough indicator cations are present

in the cation exchange resin indicator compound to be released in determinable amounts by the minimal quantity of hydrogen cations in the gastric juice. In testing for the presence of the minimal quantity in gastric juice excreted from a high acid stomach, the minimum quantity of indicator cation necessary in the ion exchange resin indicator compound may be as low as 0.02 millimole per dose of cation exchange resin indicator compound. In testing for the minimal quantity of gastric hydrochloric acid in gastric juice in stomachs having a low secretion of acid, the minimum quantity of indicator cation in the dose of cation exchange resin indicator compound will be proportionately higher. In every case, a quantity of 0.1 millimole of indicator cation in a dose of cation exchange resin indicator compound will be sufficient definitely to establish the presence of the minimal quantity of free gastric hydrochloric acid concentration.

The most important determination in an average gastric analysis is the estimation of the gastric acidity. The secretion of gastric hydrochloric acid by the parietal cells is constant. It is the volume not the concentration of parietal secretion, which varies and helps to determine the eventual concentration of the gastric acidity. Pure parietal secretion has an acidity of 165 meq./l. Yet the acid concentration of gastric contents usually is not above 60 C. U. (meq./l.). This is due to the changing proportion of parietal to non-parietal secretion as mucus, pepsin, etc.

The volume of gastric juice is generally parallel to the hydrochloric acid concentration determined therein. This is so because most of the water in gastric juice is secreted by the parietal cells. A high volume is generally accompanied by a high acid concentration. The volume, however, is not necessarily a direct measurement or indication of the acid concentration because duodenal juice, saliva and variations in mucus secretion affect the volume and thus indirectly the concentration of the hydrochloric acid.

Gastric acidity values are subdivided into four clinically significant ranges depending upon the meq./l. (clinical units) of HCl output; hyperacidity [50–100 meq. HCl/l. (pH 1.3–1.0)]; normal acidity [15–50 meq. HCl/l. (pH 1.8–1.3)]; low acidity [1–15 meq. HCl/l. (pH 3.5–1.8)]; achlorhydria [less than 1 meq. HCl/l. (pH 3.5–8.5)].

The displacement of the indicator cations from the indicator resin compound depends upon the hydrogen ion concentration (pH) as well as the quantity of the compound and the volume of solution to which the indicator compound is subjected. The average normal adult human stomach secretes about 2500 cc. of gastric juice in 24 hours under normal conditions. The range of secretion for a specific hour will depend upon certain conditions using appropriate stimulants such as caffeine sodium benzoate, histamine, etc. The average normal adult human stomach will secrete a volume of gastric juice ranging from 30 to 200 ml. averaging usually between 50 and 100 ml. Stomachs that secrete very little or no acid secrete on the average less than about 30 to 50 ml. of gastric juice in one hour after stimulation. Accordingly, on the basis of known acid secretion, an appropriate standardized unit has been predicted and administered to release a predetermined amount of indicator ion displaced by the hydrogen ions. The amount of these ions excreted in the urine will be an indication of the hydrogen ion concentration (pH) range which is characteristic of the indicator resin composition.

In vivo studies have shown us that the cation exchange resin indicator compound of this invention is administered best in a 2 gram dose for a given test of the presence of a minimal quantity of hydrogen cations in the gastric juice. This 2 gram dose is preferably made up of cation exchange indicator compounds containing preferably at least approximately 0.1 millimole of the indicator cation per dose of cation exchange resin compound and not less than 0.02 and not more than 0.50 millimole of cation indicator per dose of cation exchange resin compound.

Particularly useful indicator ion components are dyes or such ions which can be detected by a color change and which conform to the requirements above mentioned, i. e. non-toxic, not too readily displaced by any cations other than those associated with the HCl of the stomach, readily absorbed and excreted in the urine. Such dyes or ions are detected quantitatively by colorimetry or by comparison with appropriate color standards. This makes it possible to determine the amount of dye or ion excreted in the urine, present in blood, etc. within a certain predetermined time after the oral administration of the indicator resin compound. Gastric secretion may be stimulated using standard reagents such as caffeine sodium benzoate, alcohol, histamine, etc. Conditions of the test are standardized to provide reproduceable and accurate quantitative results.

Both the azo and quinone-imine group of dyes have been used as the indicator ions in preparing these compounds. Particular desirable dye indicator ions for colorimetric gastric acidity determinations, avoiding the inconvenience and disadvantage of intubation are certain of the basic dyes of the quinone-imine groups, the indamines. More specifically, the methylated amino derivatives of thiazins and their homologues which are substituted with two amino groups but the basicity of said amino groups being reduced by methyl substitution on the nitrogen atoms of said amino groups. These are symmetrical and asymmetrical methyl substituted thionins or their homologues free from solubilizing groups or acidic or basic reacting groups attached to the thionin nucleus. These basic dyes are blue, have a spectrophotometric absorption maxima in the range of about 610–670 millimicrons and are very well known as biological stains, see Conn, "Biological Stains," published by Biotech Publications, fifth edition, pages 90 through 95, 97 and 98.

These dyes are preferably used in the form of the chloride and include azure A, asymmetrical dimethyl thionin, azure C, monomethyl thionin, azure B, trimethyl thionin, Methylene Blue, tetramethyl thionin, as well as methyl homologues, such as Toluidine Blue O, Color Index No. 925. Also included are mixtures of these such as polychrome methylene blue, methylene azure, azure I and azure II, in which mixtures varying amounts of azure A and/or azure B are present.

The tetramethyl substituted thionin, methylene blue, which is typical of the entire class, is perhaps the best known non-toxic basic staining dye. Methylene blue is so readily oxidized that the presence of the azure (azure A), the lower methylated substitution products produced by such oxidation is universally recognized (see Conn). The presence of azure A in Stain Commission acceptable or medical grade USP methylene blue chloride, particularly where the methylene blue chloride solutions have been standing for some time accounts not only for some of the valuable staining properties of this preparation but also for improved gastric acidity determinations due to the presence of this material in the methylene blue ion exchange resin compounds of the present invention.

In order that this invention may be more fully understood, the following examples are given by way of illustration, but it is to be understood that the invention is not limited thereto as will be more specifically pointed out hereinafter.

EXAMPLE I

The cation resin Amberlite IRC–50 (XE–96) in the hydrogen form is treated with an aqueous solution of a suitable sodium salt or sodium hydroxide and then restored to the hydrogen cycle by eluting with hydrochloric or sulphuric acid. In the case of the methylene blue indicator exchange compound the cation exchange resin is conditioned as follows:

1. A measured amount of the cation exchange resin

Amberlite XE–96 is placed in distilled water for 24 hours, using 100 grams of resin to 225 ml. of water.

2. The resin and water mixture is poured into a glass column at the bottom of which is a perforated porcelain plate covered with nylon. The water is drawn off to one-half inch above the resin. Two percent HCl is added and allowed to run through at the rate of 70 ml. per minute until the percolate has a pH of 2 or less.

3. The column is then washed with distilled water until the pH of the percolate rises to between a pH of 4–5.

4. One percent sodium hydroxide is added until the percolate reaches a pH of 10 or more.

5. The column is again washed with distilled water until the pH of the percolate is between 8.5–9.0.

6. Two percent HCl is added until the pH becomes 2 or less and then the column is washed with distilled water until the pH rises to between 2.5 and 3.5.

The resin is now conditioned and ready for the methylene blue indicator cations. "MB" will be used to indicate the tetramethylaminophenothiazinium ion. Using the batch process the methylene blue indicator compound is made as follows:

1. Methylene blue hydrochloride is dissolved in distilled water and added to the conditioned resin so that the desired amount of methylene blue cations are available for each gram of resin. This is stirred for six hours.

2. The solution is decanted off and the resin compound is thoroughly washed with distilled water until no more dye is present in the wash.

3. The resulting methylene blue exchange indicator compound is dried at room temperature. Each gram of this methylene blue exchange indicator compound contains the desired amount of methylene blue per gram of indicator compound.

We have prepared and tested methylene blue resin compounds containing 34.5 milligrams, 65.3 milligrams, and 68.5 milligrams per gram of resin.

These compounds were tested with dilute solutions of HCl as well as with Ringer's solution and alcohol. The amount of dye displaced by the hydrogen ions of the HCl depended upon the hydrogen ion concentration. An insignificant amount of dye is displaced at a pH above 3 and also by the cations in Ringer's solution.

EXAMPLE II

A cation exchange resin made up of a granular copolymer of methacrylic acid and divinyl benzene hereinafter referred to as Amberlite XE–96 is first conditioned and then treated with azure A (C_{14}, $H_{14}N_3$, SCl) (molecular weight 291.8), for the preparation of an azure A ion exchange compound of this invention. Azure A cations are substituted for the quininium or methylene blue cations in an amount that will allow one gram of the azure A indicator compound to contain the amount of azure A desired per gram of azure A indicator compound as already stated.

Compounds were made containing both 45 mg. and 58 mg. per gram of resin (weight on air-dry basis).

The azure A indicator compound (45 mg. of azure A per gram of resin) was then placed in dilute HCl solutions of varying hydrogen ion concentration as well as in Ringer's solution in the following manner: One-tenth gram of the azure A indicator compound was placed in each of 13 test tubes, to 11 of which were added 10 ml. of HCl solution of varying hydrogen ion concentration. To each of the remaining 2 test tubes 10 ml. of distilled water and of Ringer's solution were added, respectively. The temperature was kept constant at 37.5° C. and the mixtures were stirred for 30 minutes by a slow stream of air. The amount of azure A eluted was determined with a Beckmann colorimeter.

The following table reveals the amount of axure A that was eluted from one gram of the azure A indicator compound subjected to 100 ml. of each of these solutions:

Table I

pH of HCl Solution	Amount in mg. of Azure A cations displaced from 1 gram of compound per 100 ml. of solution
5	5.7
8	8.2
1.01	8.0
1.50	13.4
1.99	8.0
2.31	4.25
2.72	1.65
3.00	0.91
3.20	0.71
3.52	0.37
4.00	0.10
H_2O	0.00
Ringer's solution	0.72

In preliminary clinical study the excretion time of the azure A dye was determined by measuring the amount of the dye excreted in urines obtained one hour and two hours following the oral administration of azure A in 20 or 40 mg. doses. The amount of azure A in the total two-hour excretion of 18 patients was found to vary from 7 to 26 percent with an average of approximately 15 percent. From this excretion factor and from the amount of azure A displaced in vitro by hydrochloric acid and by Ringer's solutions, a dose of 2 grams of the azure A resin compound was predicted as the most appropriate dose.

Definitive evaluation of the azure A indicator compound by us resulted from the clinical trial in 279 individuals at the established dose level of 2 grams. The gastric secretory response of 100 of these individuals to caffeine was determined by intubation. The gastric secretory response of the remaining 179 individuals was determined by the quininium resin indicator compound. On the trial day, breakfast was omitted. A glass of water containing 500 mgs. of caffeine sodium benzoate was administered to stimulate gastric secretion, and a control urine was collected one hour thereafter. The azure A indicator compound was administered in water and urines were collected one and two hours or only two hours thereafter. The amount of azure A present in the first and second hour excretions or in the total two-hour urine excretion was estimated both by the use of a Beckmann spectrophotometer at 620 millimicrons and by means of simple colorimetric comparator standards.

The interpretation of the results obtained with the azure A resin compound containing 45 mg. of azure A per gram of resin is summarized as follows:

Free Gastric HCl Secretion	Mg.[1] of Azure A in 2-Hour Urine
Present	0.6 or more.
Absent	0.3 or less.
Borderline	0.3–0.6.

[1] This will depend upon the amount of azure A per gram of resin.

Table 2 is a summary of the results in the 279 individuals tested with azure A resin compounds. The five discrepancies can be explained on the physiological bases previously described.

Table 2

TUBELESS GASTRIC ANALYSES WITH AZURE A RESIN COMPOUND

Control Group	No. of Indiv.	Results	
		Consistent	Non-Consistent
Free Acid	208	206	2
Achlorhydric	57	55	2
Intermittent or Borderline Secretors	9	9	0
Sub-total Gastrectomy	5	4	1
Total	279	274	5

PROCEDURE OF THE TUBELESS GASTRIC ANALYSIS TECHNIQUE

In performing these tests, the following procedure is used. The individual takes no food after midnight. At a designated hour the next morning the individual urinates and saves this urine for a control. The individual then drinks a glass of water containing the gastric stimulant such as caffeine sodium benzoate. One hour after drinking the water with the stimulant, the individual urinates and saves the urine as a second control. He then takes the ion exchange resin indicator compound in about one-half glass of water. The dose is stirred vigorously and then is drunk immediately. The individual then urinates 1 and 2 or just 2 hours after consuming the dose. This or these urine specimens are tested for the amount of displaced indicator ions. The time of the test may be shortened by omitting the gastric stimulant. However, the standard test with the gastric stimulant must be repeated if the abbreviated test is negative for free acid.

The gastric acidity of the individuals who were the subjects of these tests was determined by removing samples of the gastric juice from each of the individuals by the usual intubation technique. The gastric acidity tests of the samples so obtained indicated that the subjects could be divided into a group in whom no free hydrochloric acid was present in the gastric juice and a group in whom there was free hydrochloric acid in the gastric juice. Each of the subjects was tested for free hydrochloric acid in the gastric juice by use of the ion exchange indicator compound of this invention.

Thus, as we have pointed out, this invention has provided for the determination of gastric acidity by the displacement of identifiable indicator ions from the indicator resin compounds by the hydrogen ions of the HCl of the stomach. The indicator ions are detectable by what we believe to be simple and effective procedures. Within the spirit of this disclosure we, therefore, claim as our invention the following:

1. A diagnostic indicator ion exchange compound for determining without intubation whether the pH of gastric juice in a stomach is above or below a predetermined pH value, comprising a non-toxic, insoluble granulated cation exchange complex synthetic polymer resin containing from about 0.01 millimole to about 0.6 millimole of displaceable non-toxic thionin dye ions per gram of resin so as to be displaceable from the ion exchange resin predominantly by the ions from free hydrochloric acid secreted in the stomach, said dye ions being absorbed readily from the gastrointestinal tract and being easily detected by the dye color in urine or blood.

2. A compound as claimed in claim 1 wherein said dye ion is a cation from polychrome methylene blue.

3. A compound as claimed in claim 1 wherein said dye ion is the cation from azure A.

4. A compound as claimed in claim 1 wherein said dye ion is a cation from azure B.

5. A compound as in claim 2 wherein said resin is a copolymer of methacrylic acid and divinyl benzene.

6. A compound as in claim 3 wherein said resin is a copolymer or methacrylic acid and divinyl benzene.

7. A compound as in claim 4 wherein said resin is a copolymer of methacrylic acid and divinyl benzene.

8. A compound as in claim 1 wherein said anion exchange resin is a phenol aldehyde resin.

9. A compound as claimed in claim 1 wherein said dye cation is the cation from toluidine blue.

10. A compound as claimed in claim 1 wherein said dye cation is the cation from azure C.

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A Novel Family of Small Cysteine-rich Antimicrobial Peptides from Seed of *Impatiens balsamina* Is Derived from a Single Precursor Protein*

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Four closely related peptides were isolated from seed of *Impatiens balsamina* and were shown to be inhibitory to the growth of a range of fungi and bacteria, while not being cytotoxic to cultured human cells. The peptides, designated Ib-AMP1, Ib-AMP2, Ib-AMP3, and Ib-AMP4, are 20 amino acids long and are the smallest plant-derived antimicrobial peptides isolated to date. The Ib-AMPs (*I. balsamina* antimicrobial peptides) are highly basic and contain four cysteine residues which form two intramolecular disulfide bonds. Searches of protein data bases have failed to identify any proteins with significant homology to the peptides described here. Characterization of isolated cDNAs reveals that all four peptides are encoded within a single transcript. The predicted Ib-AMP precursor protein consists of a prepeptide followed by 6 mature peptide domains, each flanked by propeptide domains ranging from 16 to 35 amino acids in length. Such a primary structure with repeated alternating basic mature peptide domains and acidic propeptide domains has, to date, not been reported in plants.

An increasing number of cysteine-rich antifungal and antimicrobial peptides have been isolated from plants and in particular from plant seed. These peptides may have an important role to play in the protection of plants from microbial infection, and they could prove to be useful tools for the genetic engineering of fungal resistance in transgenic plants (1). Based on amino acid sequence homology, these peptides fall into at least six different classes. They include peptides isolated from seed of *Mirabilis jalapa* (1), *Amaranthus caudatus* (3), and *Zea mays* (4), members of the thionin family of peptides (5), members of the lipid transfer proteins (6–8), and members of the plant defensins (9–14).

From extracts of seed of *Impatiens balsamina*, we have isolated four small peptides the amino acid sequences of which are very closely related to each other but that do not resemble any peptides previously characterized from plants or other organisms. This paper describes the purification of the peptides and reports on their antimicrobial properties, in particular with respect to the inhibition of the growth of plant pathogenic fungi. Furthermore, a single class of cDNA has been identified that encodes all four members of this family of peptides as part of a preproprotein. Details of the characterization of the unusual structure of this cDNA and its products, as well as of their expression patterns, are presented.

EXPERIMENTAL PROCEDURES

Biological Materials—Seeds of *I. balsamina* were purchased from Sandeman Seeds (Pulborough, United Kingdom). Fungi and bacteria were grown and maintained as described previously (14, 15). The following fungal strains were used: *Alternaria longipes* (CBS62083); *Botrytis cinerea* (K1147); *Cladosporium sphaerospermum* (K0791); *Colletotrichum gloeosporioides* (SR24BTA); *Fusarium culmorum* (K0311); *Gloeodes pomigena* (field isolate; T. Sutton); *Gloeosporium solani* (CBS19432); *Nectria galligena* (MUCL6128); *Penicillium digitatum* (K0879); *Phialophora malorum* (field isolate; D. Sugar); *Sclerotinia sclerotiorum* (SES A); *Trichoderma viride* (K1127); and *Verticillium alboatrum* (K0937). The following Gram-positive bacterial strains were used: *Bacillus subtilis* (JHCC 55331); *Micrococcus luteus* (ATCC 9341); *Staphylococcus aureus* (ATCC 25923); *Streptococcus faecalis* (ATCC 29212); and the following Gram-negative bacterial strains: *Erwinia amylovora* (CFBP1430); *Escherichia coli* (HB101); *Proteus vulgaris* (JHCC 558711); *Pseudomonas solanacearum* (R48/a); *Xanthomonas campestris* pathovar *pelargonii* (INRA 10342); and *Xanthomonas oryzae* (ETH 698).

Extraction of Peptides—The purification of antimicrobial peptides from the basic protein fraction of *I. balsamina* seed was essentially as described previously (10). One-kilogram amounts of seed were ground in a coffee mill, and protein was extracted by stirring overnight at 4 °C in extraction buffer (10 mM Na_2HPO_4, 15 mM NaH_2PO_4, 100 mM KCl, 2 mM EDTA, pH 7). Ammonium sulfate was added to 80% relative saturation, and precipitated proteins were collected by centrifugation, resuspended in distilled water, and extensively dialyzed against distilled water using 2000-Da cutoff dialysis tubing (Sigma). The extract was adjusted to 50 mM NH_4Ac (pH 9) and passed over a Q-Sepharose Fast Flow column (12 × 5 cm, Pharmacia) equilibrated in 50 mM NH_4Ac (pH 9). The unbound fraction represents the basic protein fraction, and this was adjusted to pH 6 with acetic acid and passed over a S-Sepharose Fast Flow column (10 × 2.6 cm, Pharmacia) equilibrated in 50 mM NH_4Ac (pH 6). Bound proteins were eluted with a linear gradient of 50 mM–1.5 M NH_4Ac (pH 6) over 325 min at a flow rate of 3 ml/min. Proteins were monitored by the on-line measurement of the absorbance at 280 nm. Fractions with the highest antifungal activity were pooled for each peak and further purified by RP-HPLC[1] on a Pep-S column (C_2/C_{18} silica, 25 × 0.93 cm, Pharmacia). Peptides were eluted with linear gradients of 0.1% (v/v) trifluoroacetic acid to 99.9% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid over 100 min at a flow rate of 3 ml/min. Elution of peptides was monitored by absorbance at 210 nm.

Electrophoresis and Amino Acid Sequencing—The purified peptides were analyzed by SDS-PAGE on precast high density gels (PhastSys-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) Y14369.

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[1] The abbreviations used are: RP-HPLC, reversed phase high performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; AMP, antimicrobial peptide; Ib, *Impatiens balsamina*; SSPE, saline/sodium/phosphate/EDTA.

tem, Pharmacia), the sample buffer containing 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM EDTA, 0.005% (w/v) bromphenol blue, and 1% (w/v) dithiothreitol. Peptides were diffusion blotted onto 0.2-μm pore size nitrocellulose (Schleicher & Schuell) and visualized by silver staining (16). For N-terminal amino acid sequence analysis, peptides were reduced and cysteine residues were alkylated with 4-vinylpyridine prior to digestion with either trypsin (EC 3.4.21.4) or chymotrypsin (EC 3.4.21.1), both from Promega. Following protease digestion, peptide fragments were separated by RP-HPLC (Pep-S column, C_2/C_{18} silica, 25 × 0.93 cm, Pharmacia) and eluted with linear gradients of 0.1% (v/v) trifluoroacetic acid to 99.9% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid over 100 min at a flow rate of 3 ml/min. Peptide fragments were subjected to N-terminal sequencing by automated Edman degradation using a 477A Protein Sequencer (Applied Biosystems).

Mass Spectrometry—Matrix-assisted laser desorption/ionization-time of flight mass spectrometry was performed by M-Scan Ltd. (Ascot, UK) using a PerSeptive Biosystems Voyager™ Elite Biospectrometry ™ Research Station laser-desorption mass spectrometer coupled with Delayed Extraction™.

Antifungal and Antibacterial Assays—Antifungal and antibacterial assays were conducted as described previously (11, 14). The growth medium for the antifungal assays was either potato dextrose broth (Difco) at 12 g/liter (medium A), or medium A supplemented with 1 mM $CaCl_2$ and 50 mM KCl (medium B).

Antibacterial assays were carried out in 1% (w/v) tryptone (Sigma), 0.5% (w/v) low melting point agarose for *B. subtilis, Escherichia coli, M. luteus, Proteus vulgaris, Staphylococcus aureus,* and *Streptococcus faecalis* and in 1% (w/v) Bacto-Peptone (Difco), 0.5% (w/v) low melting point agarose for *Erwinia amylovora, Pseudomonas solanacearum, X. campestris,* and *X. oryzae*. Magainin I (Sigma) was used as a positive control in the antibacterial assays. Growth in each assay was assessed after the appropriate incubation period (48 h at 24 °C for fungi and 24 h at 28 °C for bacteria).

Human Cell Membrane Integrity Assay and Hemolytic Activity Assay—Membrane integrity of cultured human cells was tested on skin muscle diploid fibroblasts using the neutral red uptake method as described previously (10). Hemolytic activity was assayed using human blood group A erythrocytes as described previously (10) except that the erythrocytes were suspended in phosphate-buffered saline instead of 150 mM myo-inositol.

Construction and Screening of cDNA Library—Total RNA was purified from dry seed of *I. balsamina* by grinding in liquid nitrogen, extraction with phenol/cresol and phenol/chloroform, followed by lithium chloride precipitations (17). Poly(A)$^+$ RNA was isolated by a Poly(A)Ttract mRNA Isolation System utilizing magnetic beads (Promega). cDNA was synthesized using a ZAP-cDNA synthesis kit (Stratagene) and, following the ligation of *Eco*RI linkers and size fractionation on a Sephacryl S-400 column (Pharmacia), a library constructed in λ-ZAP (Stratagene). A DNA probe for screening the cDNA library was generated by performing PCR on the above seed cDNA fractions using a pair of degenerate oligonucleotide PCR primers based on reverse translation of the available amino acid sequence of Ib-AMP1 peptide. The degenerate primers utilized were IbAMP1-C (5′-GITG^T/$_C$TG^T/$_C$GGITGGGGICC-3′) and IbAMP1-B (5′-CACCAIC^T/$_G$IAC^G/$_A$CA^G/$_A$TA-3′), where I represents an inosine residue. The resulting 46-base pair PCR product was eluted from an acrylamide gel, purified using a Mermaid Kit (Bio101), and labeled with [α-^{32}P]dCTP using a Random Prime DNA Labeling Kit (Boehringer). The labeled PCR product was used to screen the cDNA library by plaque hybridization, following the transfer of near-confluent plaques to nylon membrane (Hybond-N, Amersham) and the UV cross-linking of DNA. Nonstringent hybridization was performed in 0.25% (w/v) Marvel (Premier Beverages, UK), 5× SSPE (1 × SSPE is 0.15 M NaCl, 0.01 M NaH_2PO_4, 0.001 M EDTA, pH 7.4), 0.1% (w/v) SDS at 35 °C and washing in 6 × SSC followed by 3 × SSC at 42 °C (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). A total of 12 hybridizing plaques were purified and subsequently *in vivo* excised to generate subclones in pBluescript SK (−) phagemid vectors (Stratagene). Sequencing was performed using a Sequenase kit (United States Biochemical). Nucleotide sequence assembly and analysis was performed using PC/GENE and IG-SUITE Intelligenetics software.

Northern and Western Blot Analysis—Seed representing a range of developmental stages was harvested from glasshouse-grown plants. Developing seed was arbitrarily classified into 5 stages based on morphology and size: stage 1 = white seed, ~1 mm in diameter; stage 2 = pale green/white, 2 mm; stage 3 = pale green/white dry, 3 mm; stage 4 = gray, dry, 4 mm; and stage 5 = brown seed coat, 3–5 mm. For germination studies, seed were germinated on damp filter paper at 28 °C in the dark, and material was harvested at 24-h intervals.

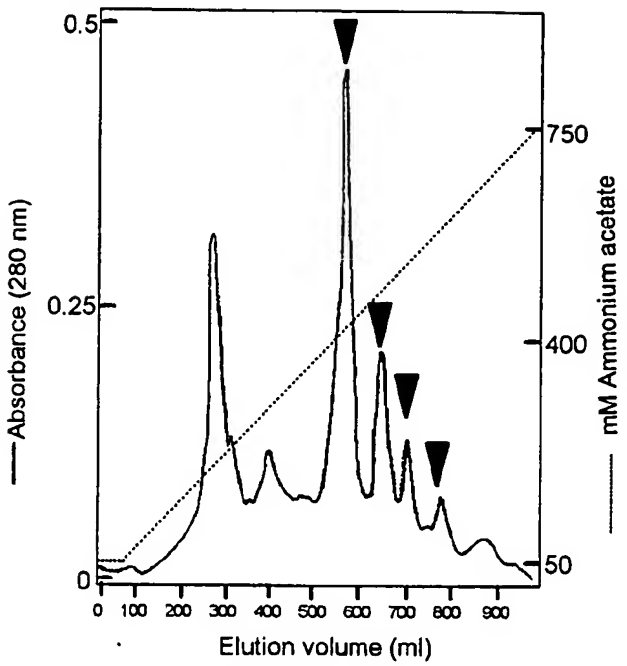

FIG. 1. **Separation of proteins in the basic protein fraction from seed of *I. balsamina*.** The basic protein fraction was loaded on a S-Sepharose high performance cation-exchange column in equilibrium with 50 mM NH_4Ac at pH 6. The column was washed with 50 mM NH_4Ac until the absorbance at 280 nm dropped below 0.01 absorbance unit, and the bound fraction eluted at 3 ml/min with a linear gradient of 50–750 mM NH_4Ac at pH 6 over 325 min. The eluate was monitored for proteins at 280 nm and collected in 10-ml fractions. Aliquots from each fraction were freeze-dried, resuspended in sterile water, and assayed for antifungal activity using a *F. culmorum* spore germination assay. Peaks of absorbance at 280 nm that correspond to fractions with antifungal activity when tested at 100 μg of total protein/ml or below in the assays are *arrowheaded*.

For Northern analysis, total RNA was isolated from seed as described by Jepson *et al.* (17) and blotted from a 1.5% (w/v) agarose gel onto Hybond-N membrane (Amersham). Hybridization was performed in 0.25% (w/v) Marvel (Premier Beverages, UK), 5 × SSPE, 0.01% (w/v) SDS at 65 °C and washing in 0.2 × SSC, 0.1% SDS at 65 °C. The [α-^{32}P]dCTP-labeled probe used for these studies was a purified DNA fragment representing the full length of the cDNA insert. The signal was detected by autoradiography.

For immunoblot analysis, total protein extracts were prepared from seed as described by Terras *et al.* (1). Two μg of total protein were electroblotted from a 15% nonreducing SDS-PAGE gel onto 0.2-μm pore size nitrocellulose membrane (Schleicher & Schuell) as described (1). The antiserum used had been raised in rabbits against bovine serum albumin-conjugated Ib-AMP1 peptide. Detection was performed using an enhanced luminescence method (Amersham) as described (1).

RESULTS

Purification and Primary Structure Determination—The basic proteins from *I. balsamina* seed were fractionated by cation exchange chromatography, and fractions assayed for antifungal activity against *F. culmorum* spores. Following chromatography, the extract yielded four peaks of activity eluting between 400 mM and 700 mM NH_4Ac (Fig. 1, *arrowheaded*). Fractions from each peak that showed the highest levels of activity were pooled and further purified by preparative RP-HPLC. Each pooled fraction yielded a single peak of absorbance at 210 nm which exactly matched the antifungal activity eluting from the column (data not shown). These active peaks were designated Ib-AMP1, Ib-AMP2, Ib-AMP3, and Ib-AMP4, respectively, according to the order of their elution from the cation exchange column.

The purified active fractions were further analyzed by SDS-

PAGE, and each was shown to contain a peptide of ~2–3 kDa (Fig. 2). The peptides ran identically whether reduced or unreduced prior to loading on the gels (data not shown), indicating that they are each composed of a single polypeptide chain.

Initial attempts to sequence the Ib-AMPs by automated Edman degradation indicated that all four peptides were N-terminally blocked. To obtain their sequences, each peptide was digested with either trypsin or chymotrypsin following modification of cysteine residues with 4-vinylpyridine, and the resulting fragments were purified by RP-HPLC prior to sequencing. The following partial amino acid sequences were generated for peptides Ib-AMP1, Ib-AMP2, Ib-AMP3, and Ib-AMP4, respectively: GRRCCGWGPGRRYCVRW*X*, GRRCCN*x*GPGRRYCKRWC, RHRCCAWGPGRKYCKRWC, and GRRCCGWGPGRRYCRRWC. Residue 7 of Ib-AMP2 could not be unambiguously identified as one of the common amino acids. Clearly, all four peptides are very close homologues of each other, with as little as one, and no more than five, amino acid differences between any pairwise alignment of the above sequences.

Electrospray Mass Spectrometry—The molecular mass of each of the purified Ib-AMP peptides was experimentally determined by electrospray mass spectrometry as 2464.6, 2527.4, 2536.6, and 2522.6 Da for Ib-AMP1, -2, -3, and -4, respectively (data not shown). These molecular mass determinations are consistent with the estimation based on SDS-PAGE.

Antifungal Activity of the Ib-AMPs—The antifungal activity of the purified peptides was assessed on 13 fungal strains, many of which are plant pathogens of significant importance to agriculture, using a standard antifungal activity assay (14). In medium A, all four peptides showed similar levels of broad spectrum activity (Table I, medium A). For the majority of the assays in this medium, the IC_{50} values were <10 μg/ml. The antifungal activity of the peptides is, however, sensitive to the ionic strength of the assay medium and in the same medium supplemented with 1 mM $CaCl_2$ and 50 mM KCl, the activity of Ib-AMP1, Ib-AMP2, and Ib-AMP3 is severely reduced (Table I, medium B). Only Ib-AMP4 maintains any significant inhibitory activity even though its activity is also markedly reduced. On some fungi, notably *F. culmorum*, the Ib-AMPs cause a very distinct swelling and hyperbranching in the spore germination assay at subinhibitory rates (Fig. 3*A*). The Ib-AMPs also inhibit the growth of germlings, and in the case of Ib-AMP4 this is also apparent in medium B (Table I). On germlings, the Ib-AMPs cause swelling and branching along the length of hyphae and at the hyphal tip (Fig. 3*B*).

Antibacterial Assays—In addition to their broad spectrum antifungal activity, the Ib-AMPs are also inhibitory to the growth of a range of bacteria, especially Gram-positive bacteria (Table II). On the Gram-positive bacteria tested, the IC_{50} values of Ib-AMP4 are lower than those obtained with the antibiotic peptide magainin I (18).

Human Cell Integrity Assays—The effect of Ib-AMP2 and Ib-AMP4 on human erythrocytes and cell cultures of human

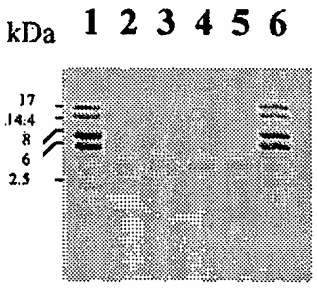

FIG. 2. **SDS-PAGE analysis of the purified Ib-AMPs.** 200 ng of each peptide were dissolved in reducing sample buffer and separated on a High Density Phastgel (Pharmacia). Peptides were transferred to nitrocellulose by capillary blotting and visualized by silver staining. *Lanes 1* and *6*, myoglobin fragments with molecular masses in kDa as indicated; *lane 2*, Ib-AMP1; *lane 3*, Ib-AMP2; *lane 4*, Ib-AMP3; *lane 5*, Ib-AMP4.

TABLE I
Antifungal activity of the Ib-AMP peptides

Protein concentrations required for 50% growth inhibition 48 h after addition were determined from dose-response curves. Proteins were added either directly to spores or to 24-h pregerminated mycelium (germlings). Assays were carried out either in medium A or in medium A supplemented with 1 mM $CaCl_2$ and 50 mM KCl (medium B).

Fungus	IC_{50}			
	Ib-AMP1	Ib-AMP2	Ib-AMP3	Ib-AMP4
	μg/ml			
Medium A, spores				
Alternaria longipes	3	12	6	3
Botrytis cinerea	12	25	6	6
Cladosporium sphaerospermum	1	6	3	1
F. culmorum	1	6	6	1
Penicillium digitatum	3	6	3	3
T. viride	6	12	12	6
V. alboatrum	3	12	6	6
Medium B, spores				
Alternaria longipes	50	>200	>200	12
Botrytis cinerea	>200	>200	>200	200
Cladosporium sphaerospermum	50	>200	100	6
F. culmorum	50	>200	100	6
Penicillium digitatum	200	>200	100	25
T. viride	>200	>200	>200	150
V. alboatrum	>200	>200	>200	50
Medium B, germlings				
Colletotrichum gloeosporioides	ND[a]	ND	ND	25
Gloeodes pomigena	ND	ND	ND	>100
Gloeosporium solani	ND	ND	ND	>100
Nectria galligena	ND	ND	ND	6
Phialophora malorum	ND	ND	ND	6
Sclerotinia sclerotiorum	ND	ND	ND	25

[a] ND, not determined.

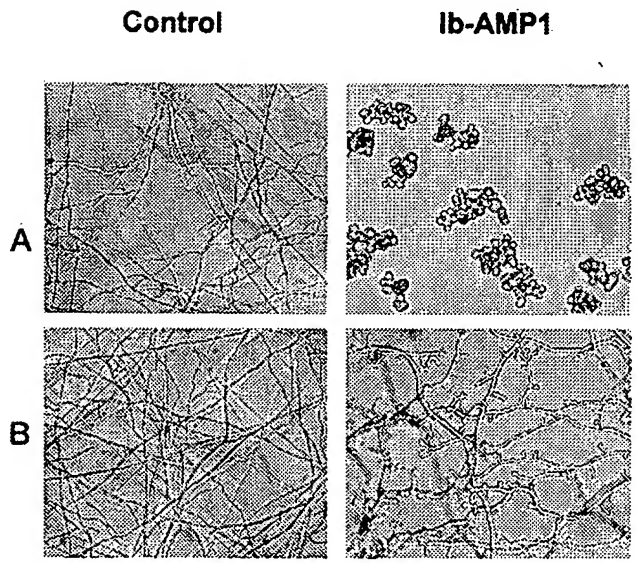

FIG. 3. **Morphological changes induced by Ib-AMP1 on partially inhibited *F. culmorum*.** Fungal spores were either germinated and grown in medium A in the presence of the peptides and observed after 24 h of incubation (*A*) or pregerminated for 24 h prior to the addition of the peptide and observed 8 h later (*B*). The concentration of Ib-AMP1 was 0.5 μg/ml in *A* and 5 μg/ml in *B*. Control incubations were carried out with the addition of water.

skin fibroblasts was investigated. At a concentration of 200 μg/ml, these peptides did not cause lysis of erythrocytes nor did they perturb membrane integrity of the cultured fibroblasts (data not shown).

cDNA Isolation and Characterization—A total of 12 cDNA clones were isolated from the dry seed cDNA library by hybrid-

TABLE II
Antibacterial activity of the Ib-AMP peptides

Protein concentrations required for 50% growth inhibition were determined after 24 h of incubation.

Bacteria	IC_{50}		
	Ib-AMP1	Ib-AMP4	Magainin I
		μg/ml	
Gram-positive bacteria			
Bacillus subtilis	10	5	20
Micrococcus luteus	10	5	20
Staphylococcus aureus	30	20	30
Streptococcus faecalis	6	5	20
Gram-negative bacteria			
Erwinia amylovora	ND[a]	>100	ND
Escherichia coli	>500	>500	ND
Proteus vulgaris	>500	>500	ND
Pseudomonas solanacearum	>500	>100	ND
X. campestris	ND	6	ND
X. oryzae	ND	15	ND

[a] ND, not determined.

```
                                M V Q K G V V F G V L L I L F I C S T L T S A D S K P
   1 ATTTTTAGGTGAGGAAAAATGGTCCAAAAAGGTGTAGTCTTTGGGGTGCTCCTAATTCTCTTCATCTGCTCTACGCTCACTTCGGCCGATTCGAAGCCAA

                N P T K E E E P A K K P D E V S V K S G G P E V S E D Q Y R H R C
 101 ACCCTACGAAAGAGGAAGAACCAGCGAAGAAACCGGATGAGGTCAGCGTAAAGAGCGGTGGACCGGAGGTGTCGGAGGATCAATACCGTCATCGGTGCTG

      A W G P G R K Y C K R W C A N A E E A A A A I P E A S E E L A Q E
 201 CGCTTGGGGACCTGGGCGAAAATATTGCAAGCGGTGGTGTGCTAACGCTGAAGAGGCGGCGGCCGCAATCCCCGAGGCAAGTGAAGAATTAGCTCAGGAG

          E A P V Y S E D Q W G R R C C G W G P G R R Y C V R W C Q N A E E
 301 GAGGCTCCGGTGTACTCGGAGGATCAGTGGGGTCGTCGGTGCTGCGGCTGGGGACCCGGCCGAAGATACTGCGTGCGCTGGTGTCAAAACGCGGAAGAGG
                                ======IbAMP1-C=====>     <=====IbAMP1-B=====
          A A A A I P E A T E K A Q E A P V Y S E D Q W G R R C C G W G P G
 401 CGGCCGCGGCAATCCCCGAGGCGACTGAAAAAGCTCAGGAGGCTCCGGTGTACTCGGAGGATCAGTGGGGTCGTCGATGCTGCGGCTGGGGACCCGGCCG

      R Y C V R W C Q N A E E A A A A V A I P E A S E K A Q E G P V Y S
 501 ACGGTATTGCGTGCGCTGGTGTCAAAACGCGGAAGAGGCGGCCGCGGCGGTGGCAATCCCCGAGGCAAGTGAGAAAGCTCAGGAGGGACCCGTGTACTCG

          E D Q W G R R C C G W G P G R R Y C V R W C S N A A D E V A T P E
 601 GAGGATCAGTGGGGTCGCCGATGCTGCGGTTGGGGACCTGGCCGTAGGTATTGCGTGCGGTGGTGCAGCAACGCCGCCGACGAGGTGGCAACACCCGAGG

          D V E P G Q Y G R R C C N W G P G R R Y C K R W C H N A A E E A T L
 701 ACGTAGAACCGGGTCAGTACGGTCGTCGGTGCTGCAACTGGGGACCTGGGCGAAGGTATTGCAAGCGGTGGTGTCATAATGCGGCTGAAGAGGCAACTCT

            K A F E E E A A R E Q P V Y S E D Q W G R R C C G W G P G R R Y C
 801 CAAGGCATTTGAAGAGGAAGCAGCTCGGGAGCAACCGGTGTACTCGGAGGACCAGTGGGGTCGCCGGTGCTGCGGTTGGGGACCCGGCCGTAGGTACTGC

      R R W C Q S A E E A A A F Q A G E V T A S L M L I M F K A C P C M
 901 AGGCGGTGGTGTCAAAGCGCCGAAGAAGCGGCTGCGTTCCAGGCTGGGGAGGTAACTGCTTCCTTGATGCTCATCATGTTTAAGGCATGCCCATGCATGG

      G P V P S V *
1001 GGCCGGTGCCTTCTGTTTAAGGCCACTCTAGCTAGCTACGTACTCTTAATAAGGGCACATGAAAAAGTTTGTCCTTTAGAAATAAGGCACAGTAAGAAAT
1101 AAAATGTCCAACTTCTTTTTATGAAAGAAGTGAACAATAAGTGTAAGCTGAATAATATATATTGTGACACGTTTGTTGTTGTACAAAAATAACATCTTTTC
1201 AGATGAACAACCTTTAATGGAAAAAAAAAAAAAAAAAA          (5)                                          \
                ~~~~~~AACTTTTATTAGTTATTA(An)          (3)                                          (1)
                ~~~~~~AACTTTTATTAGTTATTACCTA(An)      (2)
                ~~~~~~AACTTTTATTAGTTATTACCTAGA(An)    (1)
```

FIG. 4. **Complete nucleotide sequence of cDNA clone Ib22 insert and the predicted translation product.** Amino acid residues comprising the domains representing the mature Ib-AMP peptides are in *bold* and *boxed*; those comprising the predicted signal sequence are *underlined*. The predicted termination codon is *double underlined*. The DNA sequences immediately upstream of poly(A) tails in otherwise identical cDNA clones are shown. The number of independently isolated cDNA clones exhibiting these different sites of polyadenylation is indicated in *parentheses*. The annealing positions of the named degenerate oligonucleotide PCR primers used to generate the initial hybridization probe are indicated by *double arrows* underneath the first of the Ib-AMP1 repeat regions.

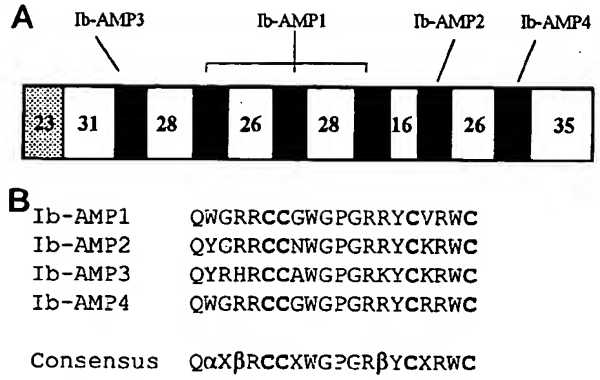

FIG. 5. **General gene structure and alignment of peptide sequences.** *A*, the generalized structure of the predicted 333 amino acid primary translation product in which the individual 20-amino acid domains representing the mature Ib-AMP peptides are *shaded*, with the Ib-AMP member indicated immediately above. The propeptide regions are *unshaded*, the predicted prepeptide (signal sequence) region is *hatched*, and the number of amino acids comprising each of these regions is indicated. *B*, amino acid sequence alignment of the predicted translation products of each mature domain. A consensus sequence for the Ib-AMPs appears below in which *upper case letters* represent invariant residues, α an aromatic residue, β a basic residue, and *X* a nonconserved residue. Cysteine residues are in *bold text*.

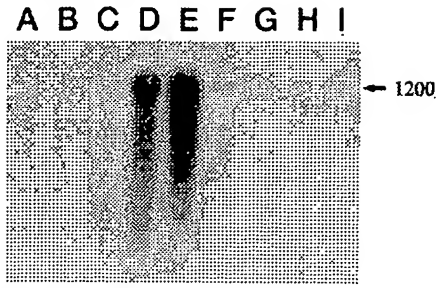

FIG. 6. **A Northern blot of total RNA from developing, mature, and germinating *I. balsamina* seed.** Total RNA isolated from developing seed was from the developmental stages described under "Experimental Procedures." Each lane on the 1.5% (w/v) agarose gel was loaded with 5 μg of total RNA. Northern blots were probed with radiolabeled fragment representing the insert of cDNA clone Ib22. The *arrow* indicates the length, in nucleotides, of the hybridizing band as determined by comparison with the migration of RNAs of known lengths (not shown). Sources of extracts on the gel were as follows: *lanes A-D*, seed at developmental stages 2–5, respectively; *lane E*, dry mature seed; *lanes F–I*, seed at 24, 48, 72, and 96 h postgermination, respectively.

ization, and the insert of the largest clone (Ib22) fully sequenced following *in vivo* excision of the phagemid. The 1238-base pair DNA sequence of the insert from this clone is presented in Fig. 4 and discussed further below. Approximately 500 base pairs of DNA sequence were obtained for each of the other cDNA clones. Although some clones appeared to be truncated at the 5′ end, the DNA sequence of each was identical to that of clone Ib22 in regions of overlaps, the only differences being apparently due to the exact position of polyadenylation. As indicated in Fig. 4, there appeared to be 5 different polyadenylation sites utilized among the 12 cDNA clones within a region spanning 52 base pairs of the 3′-untranslated region, the majority being at the same position as that of clone Ib22.

Clone Ib22 is considerably larger than would be anticipated for a cDNA encoding a single IbAMP peptide. In fact it contains an open reading frame encoding a predicted protein comprising 333 amino acids, with a molecular mass of 37,262 Da. Analysis of this predicted translation product reveals within it the presence of domains that correspond exactly in amino acid sequence to the four antimicrobial peptides described above. Ib-AMP1 is represented three times in consecutive repeats, the other Ib-AMPs once each. The six *boxed* regions in Fig. 4 represent, consecutively, Ib-AMP3, Ib-AMP1, Ib-AMP1, Ib-AMP1, Ib-AMP2, and Ib-AMP4. Hydropathy plots and sequence analysis (not shown) of the predicted translation product deriving from clone Ib22 predict a 23-amino acid N-terminal signal sequence, consistent with the fact that many plant antifungal peptides are extracellularly located (1). The general structure of the predicted translation product and an amino acid alignment of those regions assumed to encode the individual mature peptides are presented in Fig. 5.

The proprotein domains flanking each Ib-AMP peptide domain vary in length between 16 and 35 amino acids but display some degree of homology with each other, each containing at least five negatively charged amino acids, generally organized as doublets in the vicinity of the presumed cleavage sites (see Fig. 4). This composition is in contrast to the highly basic Ib-AMP domains, which are separated by these proprotein regions. Although there is no clear homology to other peptide sequences or processing sites in the data bases, it is assumed that these regions contain information required for the correct processing of the preproprotein into constituent mature peptides.

Analysis of Expression—To investigate the accumulation of Ib-AMP-related transcripts and protein during seed development, both Northern and Western blot analysis was performed on material isolated from developing, dry, and germinating seed. The hybridization pattern resulting from Northern blots probed with the entire insert of clone Ib22 is shown in Fig. 6. There appears to be hybridization to a single class of transcript of ~1200 nucleotides, which is in accordance with the size predicted from the cDNA sequence analysis of clone Ib22. The pattern of hybridization indicates that the highest accumulation of related transcript is found in dry seed (Fig. 6, *lane E*) and in the stage of development immediately prior to this (Fig. 6, *lane D*).

Western blots using Ib-AMP1 antibody indicate that immunoreactive material is most abundant in dry seed, from which the Ib-AMPs were originally isolated, and in seed undergoing the early stages of germination (Fig. 7, *lanes E, F,* and *G*). There appear to be significant quantities of such material also present in the developmental stage immediately preceding seed dry-down (Fig. 7, *lane D*). Immunoreactive material migrating at a higher position on the gel (Fig. 7, *lane C*) may represent unprocessed or incompletely processed precursor protein. An investigation into the processes involved in maturation of the precursor protein is currently under way.

DISCUSSION

Four closely related, small, basic, cysteine-rich peptides have been purified from seed of *I. balsamina* and shown to be active *in vitro* against a range of fungal and bacterial species. The majority of the amino acid sequence of the four peptides could be determined experimentally, although complete assignment was prevented because the N terminus of each was blocked. The molecular mass of Ib-AMP1 as determined by electrospray mass spectrometry indicated that the full-length Ib-AMP1 sequence was only 2 amino acids longer at its N terminus than the 18 amino acids assigned by direct amino acid sequencing. It can be predicted that the amino acid residue N-terminally adjacent to the sequenced region must be either a tryptophan or a tyrosine for chymotrypsin to have cleaved Ib-AMP1 in that position. Furthermore, based on the molecular mass estimation and the fact that the peptide is N-terminally blocked, it can be considered likely that the unassigned N-terminal residue is a cyclized glutamine. Both these speculations were indeed confirmed for all four Ib-AMP peptides by the subsequent analysis

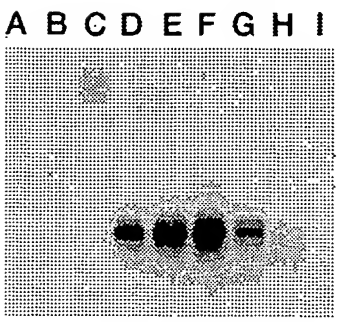

FIG. 7. **An immunoblot of protein extracts from developing, mature, and germinating *I. balsamina* seed.** Total protein was isolated from seed at the developmental stages described under "Experimental Procedures." Each lane on the 15% (w/v) nonreducing SDS-PAGE gel was loaded with 2 μg of total protein extract. Western blots were immunostained with an antiserum raised against Ib-AMP1. Sources of extracts on the gel were as follows: *lanes A–D*, seed at developmental stages 2–5, respectively; *lane E*, dry mature seed; *lanes F–I*, seed at 24, 48, 72, and 96 h postgermination, respectively.

of the cDNA clone reported here.

The molecular mass of each peptide determined experimentally by matrix-assisted laser desorption/ionization-time of flight electrospray analysis is consistently ~21 Da lower than that theoretically predicted for each by direct translation of the gene sequence. This difference is precisely accounted for if it is assumed that the N-terminal glutamine residue of each Ib-AMP peptide is cyclized and that the four internal cysteine residues form two disulfide bridges. The former supposition is supported by the fact that all classes of the Ib-AMP peptides were found to be N-terminally blocked when amino acid sequencing was attempted. The latter is supported by the fact that the native Ib-AMP peptides do not react with Ellman's free thiol reagent.

When the theoretical molecular masses for Ib-AMP1, Ib-AMP2, Ib-AMP3, and Ib-AMP4 are adjusted to take into account the N-terminal cyclization and disulfide bridge formation, the respective values of 2464.3, 2527.4, 2536.5, and 2521.4 match the experimentally determined values of 2464.6, 2527.4, 2536.6, and 2522.6 Da almost exactly. This provides further evidence that the gene represented by the Ib22 cDNA does indeed encode all four classes of isolated peptides. Furthermore, these data confirm that the unassigned residue of Ib-AMP2 is a tryptophan, as predicted from the cDNA sequence. Interestingly, a residue of the plant defensin peptide *Br*-AFP2 could not be identified by direct sequencing (11) but might reasonably be assumed to be a tryptophan residue because of close homology to other Brassicaceae antifungal proteins.

The Ib-AMP family is clearly distinct from all other families of plant antimicrobial peptides reported previously (Ref. 19; Table III). Searches of protein data bases did not identify any peptides or proteins with significant homology to either the mature Ib-AMP peptides or the intervening propeptide regions. Limited homology was noted between the Ib-AMPs and the N-terminal region of the mature peptide domain of the eight-cysteine type plant thionins, particularly with respect to the cysteine residue pattern (Table III). This might indicate an evolutionary relationship between the Ib-AMP and thionin genes involving processes of truncation and duplication. In addition, the sequence Gly-Pro-Gly-Arg-Arg-Tyr, corresponding to the conserved residues 10–15 in Ib-AMP1, Ib-AMP2, and Ib-AMP4, was identified in a number of other proteins, notably viral proteins (22). In a number of these proteins, this sequence has been shown to form a β-turn, and it is possible that a similar turn is occurring at this sequence in Ib-AMP1.

All four Ib-AMPs are inhibitory to the growth of a wide range of filamentous fungi when assayed in medium A. When this medium is supplemented with $CaCl_2$ and KCl, only Ib-AMP4 retains any significant inhibitory activity, even though it differs from the more sensitive Ib-AMP1 by only a single amino acid residue substitution. A reduction in antifungal activity in media of increased ionic strength is a common feature of most of the small cationic peptides isolated to date and probably reflects the weakening of electrostatic interactions with the target rather than any alteration of the structure of the peptide by the binding of ions from the medium (11). This is supported by the fact that the degree of reduction in antifungal activity is dependent on the test fungus (Table I). Moreover, the finding that the only one of the four Ib-AMP peptides that remains active in medium B is the most basic homologue would also support this. On the whole, the antifungal activity of the Ib-AMPs compares favorably with the more active antifungal and antimicrobial peptides purified to date from plants (2–4, 8, 10, 11).

In addition to their antifungal activity, the Ib-AMPs were also inhibitory to the growth of the four Gram-positive bacteria that were tested and to the growth of two Gram-negative *Xanthomonas* species. In these assays, their activity was compared with the antibiotic peptide magainin I (18), and on all four of the Gram-positive bacteria tested the Ib-AMPs were equally active as or more active than magainin I. Few of the other antifungal peptides isolated to date from plants show significant levels of activity on bacteria (2, 14).

From the literature, it is apparent that peptides with very different functions can share a common structure. The α-conotoxins, which have been purified from marine snails, have a similar cysteine arrangement to the Ib-AMPs, although the spacing between the two C-terminal cysteines is different (23). The solution structure for α-conotoxin GI has been determined using two-dimensional NMR and shows that the two disulfide bonds stabilize a nonrandom coil structure with two β-turns (24). Preliminary work involving the analysis of purified products released from unreduced Ib-AMP1 following digestion by trypsin showed that Cys_{16} could not be connected to Cys_{20} but rather that the C-terminal cysteine was connected to either Cys_6 or Cys_7 (data not shown). The two remaining possible combinations of pairwise connectivities (*i.e.* Cys_6-Cys_{16} and Cys_7-Cys_{20} or Cys_6-Cys_{20} and Cys_7-Cys_{16},) could not be resolved by protease digestion methods. However, NMR has since been used to determine a solution structure for Ib-AMP1, enabling a comparison with that of the α-conotoxins, and will be reported elsewhere.[2]

The amino acid sequence of each of the four Ib-AMP peptides isolated from dry seed can be identified within the predicted translation product of a single class of cDNA obtained from RNA also isolated from dry seed. One of the peptides is represented three times, the other three once. The fact that 12 individual cDNAs have essentially identical DNA sequences suggests that, at least in seed, only a single gene encoding such peptides is expressed. Southern blotting of genomic DNA suggests that there is only one gene (data not shown).

cDNAs encoding antibacterial peptides processed from a multipeptide precursor have been described previously, albeit not in plants. Inducible, proline-rich, 18-amino acid apidaecin peptides from bees are encoded by a family of cDNAs which contain up to 12 peptide repeats separated by well conserved "processing" regions (25). The cDNA sequence coding for *Xenopus* prepromagainins also has a multipeptide structure (26). In plants, there are two published examples of cDNAs encoding multipeptide precursors. The first well known example is that

[2] S. Patel and J. Thornton, personal communication.

TABLE III
Comparison of plant antifungal peptides

The size of the mature peptide (number of amino acids) and spacing of cysteine residues within its sequence is presented for Ib-AMP1 and representative members of other reported plant antifungal peptide families. Figures in the final column represent the number of amino acid residues flanking the cysteine residues indicated.

Peptide family	Representative member	Ref.	Size	Spacing of cysteine residues
Plant defensins	Rs-AFP2	1, 10	51	3-C-10-C-5-C-3-C-9-C-8-C-1-C-3-C
Knottin-type	Mj-AMP1	2	36	1-C-6-C-8-CC-3-C-10-C-3
Lipid transfer protein	Ace-AMP1	8	93	3-C-9-C-12-CC-18-C-1-C-23-C-15-C-4
Hevein-type	Ac-AMP2	3	30	3-C-4-C-4-CC-5-C-6-C-2
Macadamia	Mi-AMP1	20	76	10-C-9-C-1-C-25-C-14-C-11-C
Maize basic protein	MBP-1	4	33	6-C-3-C-13-C-3-C-4
Thionin (8-Cys type)	α-Purothionin	21	45	2-CC-7-C-3-C-8-C-3-C-1-C-8-C-6
Impatiens	Ib-AMP1	This paper	20	5-CC-8-C-3-C

of polyubiquitin cDNA, which encodes head to tail repeats of ubiquitin peptides, apparently without linker domains (27). The other example is that of a cDNA from *Nicotiana alata* encoding stigma proteinase inhibitor peptides separated from each other by dibasic dipeptide linkers (28). No structure similar to that of the Ib-AMP precursor, with acidic linker domains separating basic mature peptide domains, has yet been reported in plants. The mechanism of processing of the Ib-AMP preproprotein into its constituent mature peptides is not apparent from the inferred amino acid sequence of the translation product of the cDNA since no previously well characterized processing sites are apparent within the preproprotein on the basis of sequence homology alone. However, the presence of negatively charged amino acid doublets (EE or DE) at equivalent positions within each propeptide (including immediately upstream of five of the six mature peptide regions) might suggest the presence of specific processing sites.

The above examples of multiple mature peptides being generated from a single precursor differ in many respects from each other and from the Ib-AMP gene structure described here, possibly reflecting differences in the mechanism of processing. Nevertheless, it is reasonable to assume that concatenation of such small peptide domains is a general mechanism for enhanced simultaneous production of related peptides. Should these related peptides individually exhibit different activity profiles, such a mechanism would enable a broader activity spectrum to be achieved from a single gene transcript.

The mode of action of the Ib-AMPs is presently unknown. Of particular interest is whether the peptides are interacting directly with microbial membranes or whether they have a protein/receptor target. Even at very high rates (500 μg/ml), the Ib-AMPs do not cause any visible cell lysis or membrane collapse on fungi. The peptides were shown not to affect human cells, and they are noncytotoxic to cultured insect and plant cells (data not shown). Taken together, these preliminary data suggest that the Ib-AMPs are not acting as ionophores but rather that they are inhibiting a distinct cellular process.

As a rough estimate from the yields obtained, the Ib-AMPs account for ~0.5% of the total protein in mature *I. balsamina* seed, where they may play a role in the defense of the germinating seed. Assuming that genes in dry seed are generally transcriptionally inactive, the patterns of hybridizing mRNA revealed by Northern blot hybridization analysis might indicate that the gene is highly transcribed immediately prior to seed maturation and that the RNA remains undegraded in dry seed. Within 24 h of imbibition, the RNA level is markedly decreased, whereas protein levels are at a peak, perhaps suggesting that RNA stored in dry seed is translated and rapidly degraded at the onset of germination. It is not known whether the expression of the Ib-AMP peptides is limited to the seed, but as in the case of other plant antimicrobial peptides (1, 29) the Ib-AMPs may also be present, perhaps inducibly, in other tissues of the plant and may play a more general role in protecting the plant from microbial infections.

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Search Results - Record(s) 1 through 45 of 45 returned.

☐ 1. Document ID: US 20030096985 A1

Using default format because multiple data bases are involved.

L6: Entry 1 of 45 File: PGPB May 22, 2003

PGPUB-DOCUMENT-NUMBER: 20030096985
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030096985 A1

TITLE: Small and cysteine rich antifungal defensin and thionin-like protein genes highly expressed in the incompatible interaction

PUBLICATION-DATE: May 22, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Oh, Boung-Jun	Kwangju		KR	
Kyung Ko, Moon	Kwangju		KR	
Shin, Byongchul	Kwangju		KR	

US-CL-CURRENT: 536/23.6; 435/320.1, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 2. Document ID: US 6770750 B2

L6: Entry 2 of 45 File: USPT Aug 3, 2004

DOCUMENT-IDENTIFIER: US 6770750 B2
TITLE: Small and cysteine rich antifungal defensin and thionin-like protein genes highly expressed in the incompatible interaction

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 3. Document ID: US 6187995 B1

L6: Entry 3 of 45 File: USPT Feb 13, 2001

DOCUMENT-IDENTIFIER: US 6187995 B1
TITLE: Method for producing disease resistant plant with thionin gene from Avena sativa

Full | Title | Citation | Front | Review | Classification | Date | Reference | ... | Claims | KWIC | Draw De

☐ 4. Document ID: US 5990389 A

L6: Entry 4 of 45 File: USPT Nov 23, 1999

DOCUMENT-IDENTIFIER: US 5990389 A
TITLE: High lysine derivatives of .alpha.-hordothionin

Full | Title | Citation | Front | Review | Classification | Date | Reference | ... | Claims | KWIC | Draw De

☐ 5. Document ID: US 5885802 A

L6: Entry 5 of 45 File: USPT Mar 23, 1999

DOCUMENT-IDENTIFIER: US 5885802 A
TITLE: High methionine derivatives of .alpha.-hordothionin

Full | Title | Citation | Front | Review | Classification | Date | Reference | ... | Claims | KWIC | Draw De

☐ 6. Document ID: US 5885801 A

L6: Entry 6 of 45 File: USPT Mar 23, 1999

DOCUMENT-IDENTIFIER: US 5885801 A
TITLE: High threonine derivatives of .alpha.-hordothionin

Full | Title | Citation | Front | Review | Classification | Date | Reference | ... | Claims | KWIC | Draw De

☐ 7. Document ID: US 5703049 A

L6: Entry 7 of 45 File: USPT Dec 30, 1997

DOCUMENT-IDENTIFIER: US 5703049 A

TITLE: High methionine derivatives of .alpha.-hordothionin for pathogen-control

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 8. Document ID: US 5168066 A

L6: Entry 8 of 45 File: USPT Dec 1, 1992

DOCUMENT-IDENTIFIER: US 5168066 A
TITLE: Thionin staining and imaging technique

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 9. Document ID: US 3299035 A

L6: Entry 9 of 45 File: USPT Jan 17, 1967

DOCUMENT-IDENTIFIER: US 3299035 A
TITLE: L-aspartyl-l-alanyl-l-phenylalanyl-l-isoleucyl-glycyl-l-leucyl-l-methionin-amide and its acid addition salts [TEXT AVAILABLE IN USOCR DATABASE]

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 10. Document ID: US 2794786 A

L6: Entry 10 of 45 File: USPT Jun 4, 1957

DOCUMENT-IDENTIFIER: US 2794786 A
TITLE: Thionin dye-ion exchange resin indicator compounds [TEXT AVAILABLE IN USOCR DATABASE]

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 11. Document ID: US 2014519 A

L6: Entry 11 of 45 File: USPT Sep 17, 1935

DOCUMENT-IDENTIFIER: US 2014519 A
TITLE: Argento-chrome tetramethylthionin and process of manufacture [TEXT AVAILABLE

IN USOCR DATABASE]

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 12. Document ID: WO 132849 A2

L6: Entry 12 of 45 File: EPAB May 10, 2001

PUB-NO: WO000132849A2
DOCUMENT-IDENTIFIER: WO 132849 A2
TITLE: INHIBITORS OF VEGETABLE CYSTATHIONIN GAMMA SYNTHASE

PUBN-DATE: May 10, 2001

INVENTOR-INFORMATION:

NAME	COUNTRY
LABER, BERND	DE
CLAUSEN, TIM	DE
HUBER, ROBERT	DE
STEEGBORN, CLEMENS	DE
MESSERSCHMIDT, ALBRECHT	DE

INT-CL (IPC): C12 N 9/88; C30 B 29/58; C12 Q 1/527; A01 N 57/24; A01 N 43/76
EUR-CL (EPC): A01N043/82; A01N057/24, C12N009/88

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 13. Document ID: WO 9950390 A1

L6: Entry 13 of 45 File: EPAB Oct 7, 1999

PUB-NO: WO009950390A1
DOCUMENT-IDENTIFIER: WO 9950390 A1
TITLE: METHIONIN CONTAINING ANIMAL CELL CULTURE MEDIUM AND ITS USE

PUBN-DATE: October 7, 1999

INVENTOR-INFORMATION:

NAME	COUNTRY
JAREKRANS, MATS	SE
OLOVSSON, HANS	SE

INT-CL (IPC): C12 N 5/00; C07 K 14/555
EUR-CL (EPC): C12N005/00; C07K014/56

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 14. Document ID: EP 902089 A2

L6: Entry 14 of 45 File: EPAB Mar 17, 1999

PUB-NO: EP000902089A2
DOCUMENT-IDENTIFIER: EP 902089 A2
TITLE: Disease resistant plant including thionin gene

PUBN-DATE: March 17, 1999

INVENTOR-INFORMATION:

NAME	COUNTRY
OHASHI, YUKO	JP
MITSUHARA, ICHIRO	JP
OHSHIMA, MASAHIRO	JP
UGAKI, MASASHI	JP
HIROCHIKA, HIROHIKO	JP
HONKURA, RYOSO	JP
IWAI, TAKAYOSHI	JP
NAKAMURA, SHIGEO	JP

INT-CL (IPC): C12 N 15/82; C12 N 15/29; A01 H 5/00; A01 N 65/00
EUR-CL (EPC): A01N065/00; C07K014/415, C12N015/82 , C12N015/82

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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☐ 15. Document ID: WO 9641608 A2

L6: Entry 15 of 45 File: EPAB Dec 27, 1996

PUB-NO: WO009641608A2
DOCUMENT-IDENTIFIER: WO 9641608 A2
TITLE: PYRULARIA THIONIN CONTAINING IMMUNOTOXINS AND IMMUNOTOXIN-LIKE CONJUGATES

PUBN-DATE: December 27, 1996

INVENTOR-INFORMATION:

NAME	COUNTRY
VERNON, LEO P	
RAEL, EPPIE D	
GASANOV, SARDAR E	

INT-CL (IPC): A61 K 0/
EUR-CL (EPC): A61K035/78; C07K016/28, A61K047/48 , A61K047/48

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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☐ 16. Document ID: WO 9638563 A1

L6: Entry 16 of 45 File: EPAB Dec 5, 1996

PUB-NO: WO009638563A1
DOCUMENT-IDENTIFIER: WO 9638563 A1
TITLE: HIGH METHIONINE DERIVATIVES OF alpha -HORDOTHIONIN

PUBN-DATE: December 5, 1996

INVENTOR-INFORMATION:

NAME	COUNTRY
RAO, GURURAJ A	

INT-CL (IPC): C12 N 15/29; C12 N 15/82; C07 K 14/415; C12 N 5/10; A01 H 5/00
EUR-CL (EPC): C07K014/415; C12N015/82, C12N015/82

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 17. Document ID: WO 9638562 A1

L6: Entry 17 of 45 File: EPAB Dec 5, 1996

PUB-NO: WO009638562A1
DOCUMENT-IDENTIFIER: WO 9638562 A1
TITLE: HIGH THREONINE DERIVATIVES OF alpha -HORDOTHIONIN

PUBN-DATE: December 5, 1996

INVENTOR-INFORMATION:

NAME	COUNTRY
RAO, ARAGULA GURURAJ	US

INT-CL (IPC): C12 N 15/29; C12 N 15/82; C07 K 14/415; A01 H 5/00
EUR-CL (EPC): C07K014/415; C12N015/82

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 18. Document ID: WO 9416078 A2

L6: Entry 18 of 45 File: EPAB Jul 21, 1994

PUB-NO: WO009416078A2
DOCUMENT-IDENTIFIER: WO 9416078 A2
TITLE: HIGH LYSINE DERIVATIVES OF ALPHA-HORDOTHIONIN

PUBN-DATE: July 21, 1994

INVENTOR-INFORMATION:

NAME	COUNTRY
RAO, A GURURAJ	
BEACH, LARRY R	

INT-CL (IPC): C12N 15/29; C07K 13/00; C12N 5/10; A01H 5/00; A01N 65/00; C12N 1/21
EUR-CL (EPC): A01N065/00; C07K014/415, C12N015/82 , C12N015/82

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 19. Document ID: WO 9416078 A1

L6: Entry 19 of 45 File: EPAB Jul 21, 1994

PUB-NO: WO009416078A1
DOCUMENT-IDENTIFIER: WO 9416078 A1
TITLE: HIGH LYSINE DERIVATIVES OF ALPHA-HORDOTHIONIN

PUBN-DATE: July 21, 1994

INVENTOR-INFORMATION:

NAME	COUNTRY
RAO, A GURURAJ	
BEACH, LARRY R	

INT-CL (IPC): C12N 15/29; C07K 13/00; C12N 5/10; A01H 5/00; A01N 65/00; C12N 1/21
EUR-CL (EPC): A01N065/00; C07K014/415, C12N015/82 , C12N015/82

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 20. Document ID: EP 538193 A2

L6: Entry 20 of 45 File: EPAB Apr 21, 1993

PUB-NO: EP000538193A2
DOCUMENT-IDENTIFIER: EP 538193 A2
TITLE: Condensed cycloaliphatir-amidino-hydrazon salts as S-adenosylmethionin decarboxylase inhibitors.

PUBN-DATE: April 21, 1993

INVENTOR-INFORMATION:

NAME	COUNTRY
STANEK, JAROSLAV DR	CH
FREI, JOERG DR	CH
CARAVATTI, GIORGIO DR	CH

US-CL-CURRENT: 564/227
INT-CL (IPC): A61K 31/155; C07C 271/18; C07C 281/18
EUR-CL (EPC): C07C281/18

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 21. Document ID: EP 445434 A2

L6: Entry 21 of 45 File: EPAB Sep 11, 1991

PUB-NO: EP000445434A2
DOCUMENT-IDENTIFIER: EP 445434 A2
TITLE: Thionin stain technique.

PUBN-DATE: September 11, 1991

INVENTOR-INFORMATION:

NAME	COUNTRY
ZAHNISER, DAVID J	US
OUD, PETRUS S	US

US-CL-CURRENT: 435/34
INT-CL (IPC): G01N 1/30; G01N 21/84
EUR-CL (EPC): G01N001/30

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw De

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☐ 22. Document ID: CN 1524881 A

L6: Entry 22 of 45 File: DWPI Sep 1, 2004

DERWENT-ACC-NO: 2004-805494
DERWENT-WEEK: 200480
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Nanometer metallothionin and preparation process

INVENTOR: CUI, J; TAO, M

PRIORITY-DATA: 2003CN-0115579 (February 28, 2003)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
CN 1524881 A	September 1, 2004		000	C07K014/825

INT-CL (IPC): C07 K 14/825

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw De

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☐ 23. Document ID: US 6653463 B1

L6: Entry 23 of 45 File: DWPI Nov 25, 2003

DERWENT-ACC-NO: 2004-050525
DERWENT-WEEK: 200408
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: New nucleic acid molecule encoding a thionin-like protein with insecticidal and fungicidal activity, useful for providing pest and pathogen resistance to plants

INVENTOR: CHEN, C; CHEN, K ; KUAN, C ; LIN, C

PRIORITY-DATA: 2000US-0686332 (October 11, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 6653463 B1	November 25, 2003		011	C12N015/29

INT-CL (IPC): C12 N 15/11; C12 N 15/29; C12 N 15/63; C12 P 21/02

☐ 24. Document ID: AU 2002250729 A1, WO 200288359 A1

L6: Entry 24 of 45 File: DWPI Nov 11, 2002

DERWENT-ACC-NO: 2003-201227
DERWENT-WEEK: 200433
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: New ryegrass or fescue nucleic acid encoding a thionin, thaumatin-like, elicitor-responsive or defensin polypeptide, for modifying disease and/or plant resistance, plant defence response and/or protein storage in a plant

INVENTOR: EMMERLING, M; ONG, E K ; SAWBRIDGE, T ; SPANGENBERG, G ; SAWBRIDGE, T I

PRIORITY-DATA: 2001AU-0004735 (May 2, 2001)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 2002250729 A1	November 11, 2002		000	C12N015/29
WO 200288359 A1	November 7, 2002	E	195	C12N015/29

INT-CL (IPC): A01 H 5/00; C07 K 14/415; C07 K 14/43; C12 N 15/29

☐ 25. Document ID: US 20020150967 A1, US 6593102 B2

L6: Entry 25 of 45 File: DWPI Oct 17, 2002

DERWENT-ACC-NO: 2003-288070
DERWENT-WEEK: 200364
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Cytological staining solution useful in cytological or histological analysis, e.g. for imaging cells, comprises methanol, a phenol derivative and thionin

INVENTOR: ISENSTEIN, L M; LAPEN, D C ; MUI, K K ; SOULE, N W ; ZAHNISER, D J

PRIORITY-DATA: 2001US-0054578 (November 12, 2001), 1999US-0430116 (October 29, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 20020150967 A1	October 17, 2002		027	G01N033/48

US 6593102 B2	July 15, 2003	000	C12Q001/00

INT-CL (IPC): C12 Q 1/00; G01 N 1/30; G01 N 33/48

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | Claims | KWIC | Draw D

☐ 26. Document ID: JP 2002272292 A

L6: Entry 26 of 45 File: DWPI Sep 24, 2002

DERWENT-ACC-NO: 2002-718704
DERWENT-WEEK: 200278
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: A disease-resistant plant in which wasabi gamma-thionin gene is introduced, creation of the disease-resistant plant

PRIORITY-DATA: 2001JP-0083526 (March 22, 2001)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 2002272292 A	September 24, 2002		011	A01H005/00

INT-CL (IPC): A01 H 5/00; C07 K 14/415; C12 N 15/09

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | Claims | KWIC | Draw D

☐ 27. Document ID: NZ 524962 A, WO 200222159 A1, AU 200075771 A, EP 1322323 A1, JP 2004508412 W

L6: Entry 27 of 45 File: DWPI Dec 24, 2004

DERWENT-ACC-NO: 2002-415978
DERWENT-WEEK: 200506
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Use of thionin for e.g. stimulating immunity for the treatment of tumor development

INVENTOR: VERNON, L P

PRIORITY-DATA: 2000WO-US24947 (September 11, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
NZ 524962 A	December 24, 2004		000	A61K038/16
WO 200222159 A1	March 21, 2002	E	052	A61K038/16
AU 200075771 A	March 26, 2002		000	A61K038/16
EP 1322323 A1	July 2, 2003	E	000	A61K038/16
JP 2004508412 W	March 18, 2004		095	A61K038/00

INT-CL (IPC): A61 K 38/00; A61 K 38/16; A61 K 38/17; A61 K 38/19; A61 K 38/20; A61

K 39/00; A61 K 39/39; A61 K 45/00; A61 P 35/00; A61 P 35/04; A61 P 37/04; C07 K 14/415

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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☐ 28. Document ID: CZ 200002680 A3

L6: Entry 28 of 45 File: DWPI Mar 13, 2002

DERWENT-ACC-NO: 2002-341029
DERWENT-WEEK: 200238
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Cyclic alkythiopeptides represents anti-oxidant compound which is protected from oxydisation by amino acid methionin - NoAbstract

INVENTOR: FRIC, P; KASAFIREK, E ; TOMASOVA, H

PRIORITY-DATA: 2000CZ-0002680 (July 21, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
CZ 200002680 A3	March 13, 2002		000	C07K005/12

INT-CL (IPC): A61 K 38/12; A61 P 9/10; A61 P 11/00; A61 P 25/16; A61 P 25/28; A61 P 27/12; A61 P 29/00; C07 K 5/12

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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☐ 29. Document ID: US 6770750 B2, EP 1101771 A1, US 6300489 B1, US 20030096985 A1

L6: Entry 29 of 45 File: DWPI Aug 3, 2004

DERWENT-ACC-NO: 2001-357927
DERWENT-WEEK: 200451
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: New pathogen induced genes (pepper defensin protein gene and pepper thionin-like protein gene) from Capsicum annuum, useful for producing transgenic plants with enhanced resistance against phytopathogens, e.g. fungi or nematode

INVENTOR: CHUNG, C H; KO, M K ; OH, B ; SHIN, B ; KYUNG KO, M

PRIORITY-DATA: 1999EP-0309059 (November 15, 1999), 1999US-0442631 (November 18, 1999), 2001US-0854562 (May 15, 2001)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 6770750 B2	August 3, 2004		000	C07H019/04
EP 1101771 A1	May 23, 2001	E	022	C07K014/415
US 6300489 B1	October 9, 2001		000	C07H021/04
US 20030096985 A1	May 22, 2003		000	C07H021/02

INT-CL (IPC): A01 N 43/04; A61 K 31/70; C07 H 19/04; C07 H 21/02; C07 H 21/04; C07 K 14/415; C12 N 15/00; C12 N 15/09; C12 N 15/63; C12 N 15/70; C12 N 15/74; C12 N 15/82

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | Claims | KWIC | Draw. D

☐ 30. Document ID: WO 200133192 A1, AU 200112421 A

L6: Entry 30 of 45 File: DWPI May 10, 2001

DERWENT-ACC-NO: 2001-355359
DERWENT-WEEK: 200364
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Cytological staining solution for staining cells useful for cytological or histological analysis, comprises methanol, phenol and thionin

INVENTOR: ISENSTEIN, L M; LAPEN, D C ; MUI, K K ; SOULE, N W ; ZAHNISER, D J

PRIORITY-DATA: 1999US-0430198 (October 29, 1999), 1999US-0430116 (October 29, 1999), 1999US-0430117 (October 29, 1999), 1999US-0430196 (October 29, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 200133192 A1	May 10, 2001	E	044	G01N001/30
AU 200112421 A	May 14, 2001		000	G01N001/30

INT-CL (IPC): G01 N 1/30

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | Claims | KWIC | Draw. D

☐ 31. Document ID: JP 2004041209 A, EP 902089 A2, JP 11075594 A, AU 9883160 A, CN 1214195 A, AU 718210 B, US 6187995 B1, EP 902089 B1, DE 69820180 E

L6: Entry 31 of 45 File: DWPI Feb 12, 2004

DERWENT-ACC-NO: 1999-169239
DERWENT-WEEK: 200413
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Disease-resistant transgenic plants - expressing a thionin gene from Avena sativa

INVENTOR: HIROCHIKA, H; HONKURA, R ; IWAI, T ; MITSUHARA, I ; NAKAMURA, S ; OHASHI, Y ; OHSHIMA, M ; UGAKI, M

PRIORITY-DATA: 1997JP-0243229 (September 8, 1997), 2003JP-0273117 (July 10, 2003)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 2004041209 A	February 12, 2004		017	A01H005/00
EP 902089 A2	March 17, 1999	E	018	C12N015/82
JP 11075594 A	March 23, 1999		012	A01H005/00

AU 9883160 A	March 18, 1999		000	A01H005/00
CN 1214195 A	April 21, 1999		000	A01H001/00
AU 718210 B	April 13, 2000		000	A01H005/00
US 6187995 B1	February 13, 2001		000	C12N015/82
EP 902089 B1	December 3, 2003	E	000	C12N015/82
DE 69820180 E	January 15, 2004		000	C12N015/82

INT-CL (IPC): A01 H 1/00; A01 H 5/00; A01 N 65/00; C12 N 5/10; C12 N 15/09; C12 N 15/29; C12 N 15/63; C12 N 15/82

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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☐ 32. Document ID: WO 9902960 A1, JP 2001509591 W, AU 9882029 A, US 5942410 A, EP 996852 A1

L6: Entry 32 of 45 File: DWPI Jan 21, 1999

DERWENT-ACC-NO: 1999-121092
DERWENT-WEEK: 200147
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: DNA staining composition with improved stability - comprises a cationic stain, especially thionin, ametabisulfite and an aqueous alcoholic solution comprising methanol or ethanol

INVENTOR: GARNER, D M; LAM, P P ; PALCIC, B ; PAYNE, P W ; PAYNE, P

PRIORITY-DATA: 1997US-0888434 (July 7, 1997)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9902960 A1	January 21, 1999	E	023	G01N001/30
JP 2001509591 W	July 24, 2001		025	G01N033/48
AU 9882029 A	February 8, 1999		000	
US 5942410 A	August 24, 1999		000	A61K009/44
EP 996852 A1	May 3, 2000	E	000	G01N001/30

INT-CL (IPC): A61 K 9/44; A61 K 31/54; C09 B 55/00; C12 Q 1/68; G01 N 1/30; G01 N 33/48; G01 N 33/50; G01 N 33/53

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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☐ 33. Document ID: US 5703049 A

L6: Entry 33 of 45 File: DWPI Dec 30, 1997

DERWENT-ACC-NO: 1998-076460
DERWENT-WEEK: 199807
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Killing and inhibiting phytopathogenic microorganisms - by expressing methionine rich alpha-hordothionin, useful in, e.g. improving plant feed formulations

INVENTOR: RAO, A G

PRIORITY-DATA: 1996US-0608786 (February 29, 1996)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 5703049 A	December 30, 1997		006	A61K038/00

INT-CL (IPC): A61 K 38/00

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 34. Document ID: JP 09241252 A

L6: Entry 34 of 45 File: DWPI Sep 16, 1997

DERWENT-ACC-NO: 1997-539743
DERWENT-WEEK: 199750
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: New 3,4,6,7-tetra:hydro-1H-5,2-benzoxathionin derivatives - used as angiotensin converting enzyme and acid secretion inhibitors

PRIORITY-DATA: 1996JP-0070887 (March 4, 1996)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 09241252 A	September 16, 1997		004	C07D327/02

INT-CL (IPC): A61 K 31/38; C07 D 327/02

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 35. Document ID: WO 9641608 A2, WO 9641608 A3, AU 9659817 A

L6: Entry 35 of 45 File: DWPI Dec 27, 1996

DERWENT-ACC-NO: 1997-065280
DERWENT-WEEK: 199722
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: New target specific toxins, partic for cancer cells - comprising a molecule capable of specific binding to the surface of a cell linked to Pyrularia thionin peptide.

INVENTOR: GASANOV, S E; RAEL, E D ; VERNON, L P

PRIORITY-DATA: 1995US-0479799 (June 7, 1995)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9641608 A2	December 27, 1996	E	052	A61K000/00
WO 9641608 A3	February 6, 1997		000	A61K000/00

AU 9659817 A	January 9, 1997	000	A61K039/00

INT-CL (IPC): A61 K 0/00; A61 K 39/00

☐ 36. Document ID: CN 1192239 A, WO 9638563 A1, AU 9659611 A, EP 832235 A1, US 5885802 A, BR 9609299 A, JP 11506329 W, HU 9900878 A2, AU 707354 B, MX 9709352 A1

L6: Entry 36 of 45 File: DWPI Sep 2, 1998

DERWENT-ACC-NO: 1997-034376
DERWENT-WEEK: 200276

TITLE: New modified alpha-hordothionin having methionine amino acid substns. - to increase the methionine content of e.g. animal feed

INVENTOR: RAO, G A; RAO, A G

PRIORITY-DATA: 1995US-0460440 (June 2, 1995), 1997US-0824382 (March 26, 1997)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
CN 1192239 A	September 2, 1998		000	C12N015/29
WO 9638563 A1	December 5, 1996	E	021	C12N015/29
AU 9659611 A	December 18, 1996		000	C12N015/29
EP 832235 A1	April 1, 1998	E	000	C12N015/29
US 5885802 A	March 23, 1999		000	C12N015/09
BR 9609299 A	May 11, 1999		000	C12N015/29
JP 11506329 W	June 8, 1999		020	C12N015/09
HU 9900878 A2	July 28, 1999		000	C12N015/29
AU 707354 B	July 8, 1999		000	C12N015/29
MX 9709352 A1	February 1, 1998		000	C12N015/09

INT-CL (IPC): A01 H 1/00; A01 H 5/00; C07 K 14/00; C07 K 14/415; C12 N 5/10; C12 N 15/09; C12 N 15/29; C12 N 15/82; C12 P 21/02; C12 P 21/02; C12 R 1:91

☐ 37. Document ID: CN 1192238 A, WO 9638562 A1, AU 9659610 A, EP 828835 A1, US 5885801 A, BR 9609200 A, AU 705933 B, HU 9900876 A2, JP 11511007 W, MX 9709351 A1

L6: Entry 37 of 45 File: DWPI Sep 2, 1998

DERWENT-ACC-NO: 1997-034375
DERWENT-WEEK: 200276

TITLE: New modified alpha-hordothionin having threonine amino acid substns. - to increase the threonine content of e.g. animal feed

INVENTOR: RAO, G A; RAO, A G

PRIORITY-DATA: 1995US-0459180 (June 2, 1995), 1997US-0824379 (March 26, 1997)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
CN 1192238 A	September 2, 1998		000	C12N015/29
WO 9638562 A1	December 5, 1996	E	019	C12N015/29
AU 9659610 A	December 18, 1996		000	C12N015/29
EP 828835 A1	March 18, 1998	E	000	C12N015/29
US 5885801 A	March 23, 1999		000	C12N015/09
BR 9609200 A	May 11, 1999		000	C12N015/29
AU 705933 B	June 3, 1999		000	C12N015/29
HU 9900876 A2	July 28, 1999		000	C12N015/29
JP 11511007 W	September 28, 1999		018	C12N015/09
MX 9709351 A1	February 1, 1998		000	C12N015/29

INT-CL (IPC): A01 H 1/00; A01 H 5/00; C07 K 14/00; C07 K 14/415; C12 N 1/21; C12 N 5/10; C12 N 15/09; C12 N 15/29; C12 N 15/82; C12 P 13/08; C12 P 13/08; C12 R 1:91

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | [illegible] | Claims | KWIC | Draw. D

☐ 38. Document ID: JP 08266279 A, JP 2775405 B2

L6: Entry 38 of 45 File: DWPI Oct 15, 1996

DERWENT-ACC-NO: 1996-512666
DERWENT-WEEK: 199833
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: DNA encoding novel Avena sativa L derived thionin - which can be introduced into plant to confer high antimicrobial activity

PRIORITY-DATA: 1995JP-0074158 (March 30, 1995)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 08266279 A	October 15, 1996		011	C12N015/09
JP 2775405 B2	July 16, 1998		011	C12N015/09

INT-CL (IPC): A01 H 5/00; A01 N 63/00; A61 K 38/00; C07 H 21/04; C07 K 14/415; C12 N 5/10; C12 N 15/09; C12 P 21/02; C12 P 21/02; C12 R 1:91; C12 N 5/10; C12 R 1:91; C12 P 21/02; C12 R 1:91

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | [illegible] | Claims | KWIC | Draw. D

☐ 39. Document ID: WO 9416078 A1, DE 69428290 E, AU 9461622 A, WO 9416078 A3, EP 745126 A1, US 5990389 A, CA 2161881 C, EP 745126 B1

L6: Entry 39 of 45 File: DWPI Jul 21, 1994

DERWENT-ACC-NO: 1994-249225
DERWENT-WEEK: 200169
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Derivatives of alpha hordothionin - have high lysine content, and retain antifungal activity of parent compound

INVENTOR: BEACH, L R; RAO, A G ; BEACH, L ; RAO, A

PRIORITY-DATA: 1993US-0003885 (January 13, 1993), 1995US-0369975 (January 6, 1995), 1995US-0575654 (December 20, 1995), 1997US-0838763 (April 10, 1997)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9416078 A1	July 21, 1994	E	027	C12N015/29
DE 69428290 E	October 18, 2001		000	C12N015/29
AU 9461622 A	August 15, 1994		000	C12N015/29
WO 9416078 A3	September 1, 1994		000	C12N015/29
EP 745126 A1	December 4, 1996	E	000	C12N015/29
US 5990389 A	November 23, 1999		000	A01H005/00
CA 2161881 C	March 27, 2001	E	000	C12N015/29
EP 745126 B1	September 12, 2001	E	000	C12N015/29

INT-CL (IPC): A01 G 13/00; A01 H 5/00; A01 N 37/18; A01 N 43/36; A01 N 65/00; C07 K 13/00; C07 K 14/00; C07 K 14/415; C12 N 1/21; C12 N 5/10; C12 N 15/29; C12 N 15/82

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | Claims | KWIC | Draw De

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☐ 40. Document ID: JP 06070694 A

L6: Entry 40 of 45 File: DWPI Mar 15, 1994

DERWENT-ACC-NO: 1994-123292
DERWENT-WEEK: 199415
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Cpd. feed for flatfish using vegetable protein - comprises soybean protein-contg. vegetable protein, methionin and lysine

PRIORITY-DATA: 1992JP-0254129 (August 29, 1992)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 06070694 A	March 15, 1994		007	A23K001/14

INT-CL (IPC): A23K 1/14; A23K 1/16; A23K 1/18

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | Claims | KWIC | Draw De

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☐ 41. Document ID: FR 2183550 A, SU 688117 A

L6: Entry 41 of 45 File: DWPI Jan 25, 1974

DERWENT-ACC-NO: 1974-11878V
DERWENT-WEEK: 200394
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Deodourising residual sodium sulphate - from ethionin mfr by treatment with hydrogen peroxide stabilised with alkali phosphate

PRIORITY-DATA: 1972FR-0016637 (May 10, 1972)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
FR 2183550 A	January 25, 1974		000	
SU 688117 A	September 25, 1979		000	

INT-CL (IPC): C01D 5/00

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | Claims | KWIC | Draw D

☐ 42. Document ID: JP 48044491 A, JP 81019998 B

L6: Entry 42 of 45 File: DWPI

DERWENT-ACC-NO: 1973-57061U
DERWENT-WEEK: 200402
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Simultaneous s-adenosylmethionine and glutathione - fermentative prodn - by culture of sacchoromyces yeasts on melthionin

PRIORITY-DATA: 1971JP-0079411 (October 11, 1971)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 48044491 A			000	
JP 81019998 B	May 11, 1981		000	

INT-CL (IPC): C12P 19/18; C12P 21/02; C12R 1/85

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | Claims | KWIC | Draw D

☐ 43. Document ID: US 3299035 A

L6: Entry 43 of 45 File: USOC Jan 17, 1967

US-PAT-NO: 3299035
DOCUMENT-IDENTIFIER: US 3299035 A

TITLE: L-aspartyl-l-alanyl-l-phenylalanyl-l-isoleucyl-glycyl-l-leucyl-l-methionin-amide and its acid addition salts

DATE-ISSUED: January 17, 1967

INVENTOR-NAME: EDMOND SANDRIN; ROGER BOISSONNAS

US-CL-CURRENT: 530/329, 930/10, 930/20

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 44. Document ID: US 2794786 A

L6: Entry 44 of 45 File: USOC Jun 4, 1957

US-PAT-NO: 2794786
DOCUMENT-IDENTIFIER: US 2794786 A

TITLE: Thionin dye-ion exchange resin indicator compounds

DATE-ISSUED: June 4, 1957

INVENTOR-NAME: SEGAL HARRY L; MILLER LEON L

US-CL-CURRENT: 521/29, 436/101, 521/32, 521/33

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 45. Document ID: US 2014519 A

L6: Entry 45 of 45 File: USOC Sep 17, 1935

US-PAT-NO: 2014519
DOCUMENT-IDENTIFIER: US 2014519 A

TITLE: Argento-chrome tetramethylthionin and process of manufacture

DATE-ISSUED: September 17, 1935

INVENTOR-NAME: JULIAN BLOCK DAVID

US-CL-CURRENT: 544/4; 987/22

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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Terms	Documents
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L5: Entry 1 of 28 File: PGPB Jan 13, 2005

DOCUMENT-IDENTIFIER: US 20050009781 A1
TITLE: Methods of skin treatment and use of water-soluble beta-(1,3) glucans as active agents for producing therapeutic skin treatment agents

Detail Description Paragraph:
[0073] In addition to the primary light protection substances also secondary light protection substances of the antioxidant type find use, which interrupt the photochemichal reaction chain, which is initiated when UV radiation penetrates the skin. Typical examples of such are amino acids (e.g. glycin, histidin, tyrosin, tryptophan) and their derivatives, imidazoles (e.g. urocaninic acid) and their derivatives, peptides such as D,L-camosine, D-camosine, L-camosine and their derivatives (e.g. anserine), carotinoides, carotine (e.g. .alpha.-carotin, .beta.-carotin, lycopin) and their derivatives, chlorogenic acid and its derivatives, liponic acid and its derivatives (e.g. dihydroliponic acid), aurothioglucose, propylthiouracil and other thiols (e.g. thioredoxin, glutathion, cystein, cystin, cystamine and their glycosyl, n-acetyl, methyl, ethyl, propyl, amyl, butyl and lauryl, palmitoyl, oleyl, .gamma.-linoleyl, cholesteryl and glyceryl esters) as well as their salts, dilauryl thiodipropionate, distearyl thiodipropionate, thiodipropionic acid and their derivatives (esters, ethers, peptides, lipides, nucleotides, nucleosides and salts) as well as sulfoximine compounds (e.g. buthionin sulfoximines, homocystein sulfoximines, butionin sulfones, penta-, hexa-, hepta-thionin sufoximine) in very small compatible doses (e.g. pmol to .mu.mol/kg), further (metal) chelating agents (e.g. .alpha.-hydroxy fatty acids, palmitic acid, phytinic acid, lactoferrine), .alpha.-hydroxy acids (e.g. citric acid, lactic acid, malic acid), humin acid, gallic acid, gallic extracts, bilirubin, bifiverdin, EDTA, EGTA and their derivatives, unsaturated fatty acids and their derivatives (e.g. .gamma.-linolenic acid, linolic acid, oleic acid), folic acid and their derivatives, ubichinon and ubichinol and their derivatives, vitamin C and derivatives (e.g. ascorbyl palmitate, Mg-ascorbyl phosphate, ascorbyl acetate), tocopheroles and derivatives (e.g. vitamin E acetate), vitamin A and derivatives (vitamin A patmitate) as well as koniferyl benzoate of benzoe resin, rutinic acid and their derivatives, .alpha.-glycosylrutin, ferula acid, furfuryliden glucitol, carnosine, butylhydroxy toluene, butylhydroxy anisol, nordihydro guajak resin acid, nordihydro guajaret acid, trihydroxy butyrophenon, uric acid and their derivatives, mannose and its derivatives, super oxide dismutase, zinc and its derivatives (e.a. ZnO, ZnSO.sub.4), selen and its derivatives (e.g. selen-methionin), stilbenes and their derivatives (e.g. stilben oxide, trans-stilben oxide) and the derivatives suitable according to the invention (salts, esters, ethers, sugars, nucleotides, nucleosides, peptides and lipids) of these mentioned active substances.

L5: Entry 2 of 28 File: PGPB May 6, 2004

DOCUMENT-IDENTIFIER: US 20040087530 A1
TITLE: Methods and compositions for modulating leptin activity

Detail Description Paragraph:
[0094] In order to localize the specific anatomic regions of the hypothalamus and other parts of the brain in which leptin affects SOCS-3 mRNA levels, .sup.35S-labeled RNA antisense probe was generated. The SOCS-3A and SOCS-3B primers from above were used amplify a 450 base pair fragment of the mouse SOCS-3 CDNA. The PCR products were cloned into pCR2.1 (Invitrogen, Carlsbad, Calif.) according to the manufactures recommendations. The orientation of the cloned CDNA was verified by sequencing using standard double-stranded plasmid techniques. For generation of sense .sup.35S-labeled RNA, the plasmid was linearized by digestion with BamHI, and subjected to in vitro transcription with T7 polymerase according to the manufactures protocols (Promega). In situ hybridization histochemistry was conducted according to methods well known in the art (Simmons). Tissue sections of mouse and rat brain were mounted onto slides, air dried, and stored in desiccated boxes at -20.degree. C. Prior to hybridization, the slides were immersed in 10% neutral buffered formalin, incubated in 0.001% proteinase K (Boehringer Mannheim) for 30 min., then in 0.025% acetic anhydride for 10 min., and dehydrated in ascending concentrations ethanol. The RNA probes were then diluted to 10.sup.6 cpm/ml in hybridization solution of 50% formamide, 10 mM Tris-HCl, pH 8.0, 5 mg tRNA, 10 mM dithiothreitol, 10% dextran sulfate, 0.3 M NaCl, 1 mM EDTA, pH 8, and 1.times. Denhardt's solution (Sigma). Hybridization solution and a glass coverslip was applied to each slide and sections were then incubated for 12-16 hours at 56.degree. C. The coverslips were removed and the slides washed 4 times with 4.times.SSC. Sections were then incubated in 0.002% RNAase A (Boehringer Mannheim) with 0.5 M NaCl, 10 mM Tris-HCl, pH 8, and 1 mM EDTA, for 30 min. at 37.degree. C. Sections were rinsed in decreasing concentrations of SSC containing 0.25% DTT: 2.times. at 50.degree. C. for 1 hour, 0.2.times. at 55.degree. C. for 1 hour, and 0.2.times. for 1 hour at 60.degree. C. Sections were next dehydrated in graded ethanol (50, 70, 80, and 90%) containing 0.3 M NH.sub.4OAc followed by 100% ethanol. Slides were air dried and placed in X-ray film cassettes with BMR-2 film (Kodak) for 3-5 days. Slides were then dipped in NTB2 photographic emulsion (Kodak), dried and stored with desiccant in foil-wrapped slide boxes at 4.degree. C. for 2-3 weeks. Slides were developed with D-19 developer (Kodak), counterstained with thionin, dehydrated in graded ethanol, cleared in xylene, and coverslipped with with Permaslip. Sections were analyzed with a Zeiss Axioplan light microscope using brightfield and darkfield optics. Photomicrographs were produced by capturing images with a digital camera (Kodak, DCS) mounted directly on the microscope and an Apple Macintosh Power PC computer. Image editing software (Adobe Photoshop) was used to combine photomicrographs into plates and figures were printed on a dye sublimation printer (Kodak 8600). Only the sharpness, contrast, and brightness were adjusted. The results are shown in FIG. 2. In brain sections from normal rats fed ad libitum and given a single intravenous injection of recombinant leptin (1 .mu.g/g body weight), strong specific hybridization was detected in the arcuate nucleus (Arc) and the dorsomedial hypothalamic nucleus (DMH), as compared to saline injected rat brain sections (FIGS. 2B and 2A, respectively). In other regions of the brain, including the cerebellum, no specific hybridization signals were detected.

L5: Entry 5 of 28 File: PGPB May 15, 2003

DOCUMENT-IDENTIFIER: US 20030091555 A1
TITLE: Bactericidal composition containing peptide and chelating agent

Abstract Paragraph:
A bactericidal composition is provided, which comprises as effective ingredients (a) at least one substance selected from the group consisting of ethylenediaminetetraacetic acid and metal salts thereof and (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin. The bactericidal composition, for example, has the effect of sterilizing food poisoning bacteria at a low concentration and is highly safe.

Summary of Invention Paragraph:
[0011] The first aspect of the present invention relates to a bactericidal composition comprising as effective ingredients (a) at least one substance selected from the group consisting of ethylenediaminetetraaceti- c acid (sometimes referred to as EDTA hereinafter) and metal salts thereof and (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin.

Summary of Invention Paragraph:
[0013] The third aspect of the present invention relates to a bactericidal composition according to the second aspect of the present invention, in which the content of the above (a) at least one substance selected from the group consisting of EDTA and metal salts thereof is 0.05 mM or more and less than 1.5 mM and the content of the above (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin is 1 .mu.g/mL or more and 150 .mu.g/mL or less.

Brief Description of Drawings Paragraph:
[0014] FIG. 1 is a graph illustrating minimum bactericidal concentration (MBC) of alpha-type thionin of wheat when food poisoning bacteria are cultivated at 37.degree. C. for 1 day in an LB medium containing EDTA-2Na in a varied concentration.

Detail Description Paragraph:
[0015] The bactericidal composition of the present invention contains at least one substance selected from the group consisting of EDTA and metal salts thereof and at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin as effective ingredients.

Detail Description Paragraph:
[0027] The bactericidal composition of the present invention may be formulated into various forms. For example, EDTA or its metal salt and thionin may be simultaneously simultaneously added to various foods. EDTA and its metal salts as well as thionins have heat resistance, so that they may be added to food prior to pasteurize the food.

Detail Description Paragraph:
[0028] To sterilize food poisoning bacteria contained in food, it may be advantageous to simultaneously add 0.1 to 0.5 mM EDTA or its metal salt and 2 to

100 .mu.g/mL thionin for V. parahaemolyticus, 0.1 to 1.49 mM EDTA or its metal salt and 2 to 100 .mu.g/mL thionin for Salmonella sp., or 0.5 to 1.49 mM EDTA or its metal salt and 10 to 100 .mu.g/mL thionin for E. coli. However, the lower the concentration of EDTA or its metal salt is, the higher the concentration of thionin to be added must be.

Detail Description Paragraph:
[0030] According to the present invention, there is provided a bactericidal composition comprising as active ingredients (a) at least one substance selected from the group consisting of EDTA and metal salts thereof and (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin.

Detail Description Paragraph:
[0032] Aqueous solutions of EDTA or its metal salts and of thionin are each colorless, transparent and odorless, so that they give no influence on the taste of food and so forth.

Detail Description Paragraph:
[0040] Then, after adding predetermined amounts of thionin and EDTA, the bacteria were inoculated so that the concentration of bacteria was about 1.times.10.sup.6 cells/mL. After cultivating the bacteria for 1 day for other bacteria, the viable count was measured by a method of diluted plate culture. The concentration in which the viable count was decreased to {fraction (1/100)} or less was defined as minimum bactericidal concentration (MBC) and the concentration in which the viable count was not increased was defined as minimum inhibitory concentration (MIC). The results obtained are shown in Tables 1, 2 and 3, as well as in FIG. 1.

Detail Description Paragraph:
[0041] As will be clear from Table 1, when alpha-type thionin of wheat was added alone, the test bacteria except for Salmonella typhimurium JCM 6977 and V. parahaemolyticus IFO 12711 showed an MIC of 50 .mu.g/mL or more. On the other hand, when EDTA-2Na was added alone, Salmonella sp. and E. coli had an MBC of 10 mM or more whereas other bacteria had an MBC of 1 mM or less.

Detail Description Paragraph:
[0042] Next, 10 .mu.g/mL of alpha-type thionin of wheat and 0.02 to 10 mM of EDTA-2NA were added simultaneously. In the case of food poisoning bacteria, the MBC of EDTA-2Na was decreased as compared with the addition of EDTA-2Na alone.

Detail Description Paragraph:
[0044] Furthermore, the MBC of alpha-type thionin of wheat on S. typhimurium JCM 6977, E. coli JCM 5491 and V. parahaemolyticus IFO 12711 in the presence of EDTA-2Na in different concentrations was examined, and the results as shown in FIG. 1 were obtained. The tests were carried out by cultivation in an LB medium at 37.degree. C. for 1 day. In FIG. 1, the symbol .circle-solid. indicates S. typhimurium JCM 6977, .largecircle. indicates E. coli JCM 5491 and .quadrature. indicates V. parahaemolyticus IFO 12711. Concerning the antibacterial activity of thionin in the coexistence of EDTA-2Na, substantially no difference was observed between the thionin derived from wheat and the thionin derived from barley, and also also between the alpha-type and the beta-type (Table 3).

Detail Description Table CWU:
1 TABLE 1 MIC MBC Thionin*.sup.1 EDTA-2Na Thionin + Strain (.mu.g/mL) (mM) EDTA-2Na*.sup.2 Salmonella typhimurium 30 20 1 JCM 6977 Salmonella enteritidis 100< 50< 1 IFO 3313 Escherichia coli 01:K1:H7 100< 50 10 JCM 1649 Escherichia coli 06 100 10 1 JCM 5491 Vibrio parahaemolyticus 20 1 0.1 IFO 12711 Bacillus cereus 100 0.5 0.02 JCM 2152 Bacillus cereus 100< 0.2 0.05 IFO 15305 *.sup.1Alpha-type thionin of wheat *.sup.2MBC (mM) of EDTA-2Na in the coexistence of 10 .mu.g/mL of wheat alpha-type thionin

Detail Description Table CWU:
2TABLE 2 Thionin* MBC (nM) Strain (10 .mu.g/mL) EDTA EDTA-2Na EDTA-4Na S. typhimurium - 5 20 50< JCM 6977 + 0.1 1 1 E. coli - 5 10 10 JCM 5491 + 1 1 1
*Alpha-type thionin of wheat

CLAIMS:

1. A bactericidal composition comprising as effective ingredients (a) at least one substance selected from the group consisting of ethylenediaminetetraacetic acid and metal salts thereof and (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin.

3. The bactericidal composition according to claim 2, wherein the content of said (a) at least one substance selected from the group consisting of ethylenediaminetetraacetic acid and metal salts thereof is 0.05 mM or more and less than 1.5 mM and the content of said (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin is 1 .mu.g/mL or more and 150 .mu.g/mL or less.

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L5: Entry 6 of 28 File: PGPB Oct 3, 2002

DOCUMENT-IDENTIFIER: US 20020142466 A1
TITLE: Methods and compositions for modulating ciliary neurotrophic factor activity

Detail Description Paragraph:
[0116] In order to localize the specific anatomic regions of the hypothalamus and other parts of the brain in which leptin affects SOCS-3 mRNA levels, .sup.35S-labeled RNA antisense probe was generated. The SOCS-3 A and SOCS-3B primers from above were used amplify a 450 base pair fragment of the mouse SOCS-3 cDNA. The PCR products were cloned into pCR2.1 (Invitrogen, Carlsbad, Calif.) according to the manufactures recommendations. The orientation of the cloned cDNA was verified by sequencing using standard double-stranded plasmid techniques. For generation of sense .sup.35S-labeled RNA, the plasmid was linearized by digestion with BamHI, and subjected to in vitro transcription with T7 polymerase according to the manufactures protocols (Promega). In situ hybridization histochemistry was conducted according to methods well known in the art (Simmons). Tissue sections of mouse and rat brain were mounted onto slides, air dried, and stored in desiccated boxes at -20.degree. C. Prior to hybridization, the slides were immersed in 10% neutral buffered formalin, incubated in 0.001% proteinase K (Boehringer Mannheim) for 30 min., then in 0.025% acetic anhydride for 10 min., and dehydrated in ascending concentrations ethanol. The RNA probes were then diluted to 10.sup.6 cpm/ml in hybridization solution of 50% formamide, 10 mM Tris-HCl, pH 8.0, 5 mg tRNA, 10 mM dithiothreitol, 10% dextran sulfate, 0.3 M NaCl, 1 mM EDTA, pH 8, and 1.times. Denhardt's solution (Sigma). Hybridization solution and a glass coverslip was applied to each slide and sections were then incubated for 12-16 hours at 56.degree. C. The coverslips were removed and the slides washed 4 times with 4.times. SSC. Sections were then incubated in 0.002% RNAase A (Boehringer Mannheim) with 0.5 M NaCl, 10 mM Tris-HCl, pH 8, and 1 mM EDTA, for 30 min. at 37.degree. C. Sections were rinsed in decreasing concentrations of SSC containing 0.25% DTT: 2.times. at 50.degree. C. for 1 hour, 0.2.times. at 55.degree. C. for 1 hour, and 0.2.times. for 1 hour at 60.degree. C. Sections were next dehydrated in graded ethanol (50, 70, 80, and 90%) containing 0.3 M NH.sub.4OAc followed by 100% ethanol. Slides were air dried and placed in X-ray film cassettes with BMR-2 film (Kodak) for 3-5 days. Slides were then dipped in NTB2 photographic emulsion (Kodak), dried and stored with desiccant in foil-wrapped slide boxes at 4.degree. C. for 2-3 weeks. Slides were developed with D-19 developer (Kodak), counterstained with thionin, dehydrated in graded ethanol, cleared in xylene, and coverslipped with with Permaslip. Sections were analyzed with a Zeiss Axioplan light microscope using brightfield and darkfield optics. Photomicrographs were produced by capturing images with a digital camera (Kodak, DCS) mounted directly on the microscope and an Apple Macintosh Power PC computer. Image editing software (Adobe Photoshop) was used to combine photomicrographs into plates and figures were printed on a dye sublimation printer (Kodak 8600). Only the sharpness, contrast, and brightness were adjusted.

L5: Entry 13 of 28 File: USPT Dec 31, 2002

DOCUMENT-IDENTIFIER: US 6500410 B1
TITLE: Sun protection agents

Brief Summary Text (41):
In addition to the primary light protection substances also secondary light protection substances of the antioxidant type find use, which interrupt the photochemichal reaction chain, which is initiated when UV radiation penetrates the skin. Typical examples of such are amino acids (e.g. glycin, histidin, tyrosin, tryptophan) and their derivatives, imidazoles (e.g. urocaninic acid) and their derivatives, peptides such as D,L-camosine, D-camosine, L-camosine and their derivatives (e.g. anserine), carotinoides, carotine (e.g. .alpha.-carotin, .beta.-carotin, lycopin) and their derivatives, chlorogenic acid and its derivatives, liponic acid and its derivatives (e.g. dihydroliponic acid), aurothioglucose, propylthiouracil and other thiols (e.g. thioredoxin, glutathion, cystein, cystin, cystamine and their glycosyl, n-acetyl, methyl, ethyl, propyl, amyl, butyl and lauryl, palmitoyl, oleyl, .gamma.-linoleyl, cholesteryl and glyceryl esters) as well as their salts, dilauryl thiodipropionate, distearyl thiodipropionate, thiodipropionic acid and their derivatives (esters, ethers, peptides, lipides, nucleotides, nucleosides and salts) as well as sulfoximine compounds (e.g. buthionin sulfoximines, homocystein sulfoximines, butionin sulfones, penta-, hexa-, hepta-thionin sufoximine) in very small compatible doses (e.g. pmol to .mu.mol/kg), further (metal) chelating agents (e.g. .alpha.-hydroxy fatty acids, palmitic acid, phytinic acid, lactoferrine), .alpha.-hydroxy acids (e.g. citric acid, lactic acid, malic acid), humin acid, gallic acid, gallic extracts, bilirubin, bifiverdin, EDTA, EGTA and their derivatives, unsaturated fatty acids and their derivatives (e.g. .gamma.-linolenic acid, linolic acid, oleic acid), folic acid and their derivatives, ubichinon and ubichinol and their derivatives, vitamin C and derivatives (e.g. ascorbyl palmitate, Mg-ascorbyl phosphate, ascorbyl acetate), tocopheroles and derivatives (e.g. vitamin E acetate), vitamin A and derivatives (vitamin A patmitate) as well as koniferyl benzoate of benzoe resin, rutinic acid and their derivatives, .alpha.-glycosylrutin, ferula acid, furfuryliden glucitol, carnosine, butylhydroxy toluene, butylhydroxy anisol, nordihydro guajak resin acid, nordihydro guajaret acid, trihydroxy butyrophenon, uric acid and their derivatives, mannose and its derivatives, super oxide dismutase, zinc and its derivatives (e.g. ZnO, ZnSO.sub.4), selen and its derivatives (e.g. selen-methionin), stilbenes and their derivatives (e.g. stilben oxide, trans-stilben oxide) and the derivatives suitable according to the invention (salts, esters, ethers, sugars, nucleotides, nucleosides, peptides and lipids) of these mentioned active substances.

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L5: Entry 18 of 28 File: USPT Aug 14, 2001

DOCUMENT-IDENTIFIER: US 6274330 B1
TITLE: L-AP4 sensitive glutamate receptors

Detailed Description Text (21):
For in situ hybridization, adult Sprague-Dawley rats (200-250 g) were anesthetized with pentobarbital and perfused transcardially with ice-cold saline, followed by ice-cold fixative (4% paraformaldehyde/0.1 M sodium borate, pH 9.5). The brains were post-fixed overnight in fixative containing 10% sucrose. Sections (25 .mu.m) were mounted on gelatin- and poly-L-lysine-coated glass, fixed for 15 minutes in 4% paraformaldehyde/0.1 M phosphate buffered saline (PBS), washed twice in 0.1 M PBS, and treated for 30 min at 37.degree. C. in proteinase K (0.001% in 0.1 M Tris/0.05 M EDTA, pH 8) followed by 0.0025% acetic anhydride in 0.1 M triethanolamine at room temperature followed by dehydration. A 719 bp XhoI fragment containing nucleotides 1-197 of mGluR7 and a 1234 bp XhoI/PstI fragment of mGluR4 containing nucleotides 791-2025 were subcloned into pBluescript KS(+). .sup.35 S-labeled antisense RNA was transcribed from each template and used for hybridization at 10.sup.7 cpm/ml for 20 hours at 58.degree. C. in hybridization buffer (Mountjoy et al., Science 257: 1248-1251 [1992]). Slides were processed as described (Simerly and Young, Mol. Endocrinol. 5: 424-432 [1991]) then dipped in NTB-2 liquid photographic emulsion (Kodak), exposed for 13 days, developed with D-19 developer and counterstained with thionin.

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L5: Entry 19 of 28 File: USPT Oct 24, 2000

DOCUMENT-IDENTIFIER: US 6136550 A
TITLE: Neuronal nicotinic acetylcholihne receptor compositions

Detailed Description Text (235):
Antisense [.sup.35 S]-UTP-labeled RNA probes were synthesized in vitro from pESD77 and used to map the distribution of transcripts corresponding to .lambda.ESD-7 in the rat brain. Paraformaldehyde-fixed 30 .mu.m thick rat brain sections were mounted on polylysine coated slides, then digested with proteinase K (10 mg/ml, 37.degree. C., 30 min), acetylated and dehydrated in graded ethanol solutions. Approximately 5.times.10.sup.5 cpm/ml of the RNA probe was hybridized in situ at 55.degree. C. for 12 hrs in 50% formamide, 0.3M NaCl, 10 mM Tris (pH 8), 1 mM EDTA, 0.05% tRNA, 10% dextran sulfate, IX Denhardt's solution, and 10 mM DTT. Glass cover slips were removed from tissue sections by washing in 4x SSC for 15min at room temperature. Sections were treated with RNase A (20 .mu.g/ml, 37.degree. C., 30 min), washed for 30 min in 2x SSC, 1 mM DTT at room temperature and for 30 min in 0.1x SSC, 1 mM DTT at 55.degree. C. Sections were dehydrated in graded ethanol solutions containing 1 mM DTT and exposed to Kodak XAR film at room temperature for 1-2 days. For higher resolution analysis slides were dipped in Kodak NTB-2 nuclear photographic emulsion, which was diluted 1:1 with distilled water, at 40.degree. C. Seven to ten days after dipping, slides were developed and stained with thionin. The distribution of silver grains was analyzed with dark field illumination.

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L5: Entry 4 of 28 File: PGPB Dec 11, 2003

DOCUMENT-IDENTIFIER: US 20030228654 A1
TITLE: Method for producing antimicrobial protein and fusion protein

Detail Description Paragraph:
[0076] The insoluble fractions of the fusion proteins of the Examples 1 and 2 and the Comparative Example 2 and the insoluble fraction from the culture of Escherichia coli BL21 (DE3) pLysS with no inserted vector therein as the control for the assay of antimicrobial activity were rinsed twice in 0.5% Triton X-100/1 mM EDTA. Subsequently, a urea solution (8 M urea, 50 mM Tris-HCl at pH 8.0, 1 mM DTT, 1 1 mM EDTA) was added for solubilization. After centrifugation, the supernatant was transferred to a dialysis tube, for one-hour dialysis at 4.degree. C. against 4M urea solution. During the dialysis against the urea solution containing DTT, the disulfide bond in thionin may be cleaved.

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DATE: Thursday, February 24, 2005

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L1	thionin	662
☐	L2	$thionin	1116
☐	L3	L2 or l1	1221
☐	L4	L3 same (edta or na-edta or sodiumedta or naedta or ethylenediamine$ ir ethylene-diamine$)	27
☐	L5	L3 same (edta or na-edta or sodiumedta or naedta or ethylenediamine$ or ethylene-diamine$)	28
☐	L6	l2.ti. not l5	45
☐	L7	l6 and (edta or na-edta or sodiumedta or naedta or ethylenediamine$ ir ethylene-diamine$)	0
☐	L8	l6 and (edta or na-edta or sodiumedta or naedta or ethylenediamine$ or ethylene-diamine$)	0
☐	L9	l1.ti. and edta	2

END OF SEARCH HISTORY

NiceProt View of Swiss-Prot: P01544

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**THN1_WHEAT**
Primary accession number	**P01544**
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 01, July 1986
Sequence was last modified in	Release 26, July 1993
Annotations were last modified in	Release 46, February 2005

Name and origin of the protein

Protein name	**Alpha-1-purothionin [Precursor] [Fragment]**
Synonym	**Purothionin A-II**
Gene name	**Name: THI1.1** Synonyms: PUR-D1
From	Triticum aestivum (Wheat) [TaxID: 4565]
Taxonomy	Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Pooideae; Triticeae; Triticum.

References

[1] NUCLEOTIDE SEQUENCE.
TISSUE=Endosperm;
DOI=10.1104/pp.106.3.1221;MEDLINE=95125120;PubMed=7824649 [NCBI, ExPASy, EBI, Israel, Japan]
Castagnaro A., Marana C., Carbonero P., Garcia-Olmedo F.;
"cDNA cloning and nucleotide sequences of alpha 1 and alpha 2 thionins from hexaploid wheat endosperm.";
Plant Physiol. 106:1221-1222(1994).

[2] PROTEIN SEQUENCE OF 17-61.
STRAIN=cv. Manitoba 3;
MEDLINE=78026451;PubMed=914810 [NCBI, ExPASy, EBI, Israel, Japan]
Ohtani S., Okada T., Yoshizumi H., Kagamiyama H.;
"Complete primary structures of two subunits of purothionin A, a lethal protein for brewer's yeast

from wheat flour.";
J. Biochem. 82:753-767(1977).

[3] PROTEIN SEQUENCE OF 17-61.
Ohtani S., Okada T., Kagamiyama H., Yoshizumi H.;
"The amino acid sequence of purothionin A, a lethal toxic protein to brewer's yeast from wheat.";
Agric. Biol. Chem. 39:2269-2270(1975).

[4] PROTEIN SEQUENCE OF 17-61.
STRAIN=cv. Manitou;
Jones B.L., Mak A.S.;
"Amino acid sequences of the two alpha-purothionins of hexaploid wheat.";
Cereal Chem. 54:511-523(1977).

[5] X-RAY CRYSTALLOGRAPHY (2.5 ANGSTROMS).
MEDLINE=91045879;PubMed=2235992 [NCBI, ExPASy, EBI, Israel, Japan]
Teeter M.M., Ma X.-Q., Rao U., Whitlow M.;
"Crystal structure of a protein-toxin alpha 1-purothionin at 2.5A and a comparison with predicted models.";
Proteins 8:118-132(1990).

Comments

- ***FUNCTION***: Thionins are small plant proteins which are toxic to animal cells. They seem to exert their toxic effect at the level of the cell membrane. Their precise function is not known.
- ***SUBCELLULAR LOCATION***: Secreted.
- ***SIMILARITY***: Belongs to the plant thionin (TC 1.C.44) family [view classification].

Copyright

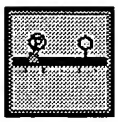
Cross-references

EMBL	X70666; CAA50004.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]
PDB	2PLH; X-ray; @=17-60.[ExPASy / RCSB / EBI]
InterPro	IPR001010; Thionin. Graphical view of domain structure.
Pfam	PF00321; Thionin; 1. Pfam graphical view of domain structure.
PRINTS	PR00287; THIONIN.
PROSITE	PS00271; THIONIN; 1.
BLOCKS	P01544.
ProtoNet	P01544.
ProtoMap	P01544.
PRESAGE	P01544.
DIP	P01544.
ModBase	P01544.
SMR	P01544; FF7310D921C4EE30.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

3D-structure; **Direct protein sequencing**; **Plant defense**; **Plant toxin**; **Signal**; **Thionin**.

Features

 Feature table viewer

 Feature aligner

Key	From	To	Length	Description
NON_TER	1	1		
SIGNAL	<1	16	>16	
CHAIN	17	61	45	Alpha-1-purothionin.
CHAIN	62	126	65	Acidic protein.
DISULFID	19	55		
DISULFID	20	47		
DISULFID	28	45		
DISULFID	32	41		
STRAND	18	20	3	
HELIX	23	32	10	
TURN	33	35	3	
HELIX	38	45	8	
TURN	46	46	1	
STRAND	47	49	3	

Sequence information

Length: **126 AA** [This is the length of the partial sequence of the unprocessed precursor]

Molecular weight: **13526 Da** [This is the MW of the partial sequence of the unprocessed precursor]

CRC64: **FF7310D921C4EE30** [This is a checksum on the sequence]

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CLLILGLVLE QLQVEGKSCC RSTLGRNCYN LCRARGAQKL CAGVCRCKIS SGLSCPKGFP

        70         80         90        100        110        120
KLALESNSDE PDTIEYCNLG CRSSVCDYMV NAAADDEEMK LYVENCADAC VSFCNGDAGL

PSLDAY
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P01544 in FASTA format

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BLAST submission on ExPASy/SIB or at NCBI (USA)

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

ScanProsite, MotifScan

Search the SWISS-MODEL Repository

NiceProt View of Swiss-Prot: Q42596

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**THN21_ARATH**
Primary accession number	**Q42596**
Secondary accession numbers	Q8LCH6 Q9C7S9
Entered in Swiss-Prot in	Release 43, March 2004
Sequence was last modified in	Release 43, March 2004
Annotations were last modified in	Release 46, February 2005

Name and origin of the protein

Protein name	**Thionin 2.1 [Precursor]**
Synonyms	None
Gene name	**Name: THI2.1** OrderedLocusNames: At1g72260 ORFNames: T9N14.13
From	Arabidopsis thaliana (Mouse-ear cress) [TaxID: 3702]
Taxonomy	Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots; rosids; eurosids II; Brassicales; Brassicaceae; Arabidopsis.

References

[1] NUCLEOTIDE SEQUENCE, TISSUE SPECIFICITY, AND INDUCTION.
STRAIN=cv. Columbia;
DOI=10.1104/pp.109.3.813;MEDLINE=96079536;PubMed=8552715 [NCBI, ExPASy, EBI, Israel, Japan]
Epple P., Apel K., Bohlmann H.;
"An Arabidopsis thaliana thionin gene is inducible via a signal transduction pathway different from that for pathogenesis-related proteins.";
Plant Physiol. 109:813-820(1995).

[2] NUCLEOTIDE SEQUENCE [LARGE SCALE GENOMIC DNA].
STRAIN=cv. Columbia;
DOI=10.1038/35048500;MEDLINE=21016719;PubMed=11130712 [NCBI, ExPASy, EBI, Israel,

Japan]
Theologis A., Ecker J.R., Palm C.J., Federspiel N.A., Kaul S., White O., Alonso J., Altafi H., Araujo R., Bowman C.L., Brooks S.Y., Buehler E., Chan A., Chao Q., Chen H., Cheuk R.F., Chin C.W., Chung M.K., Conn L., , Davis R.W.;
"Sequence and analysis of chromosome 1 of the plant Arabidopsis thaliana.";
Nature 408:816-820(2000).

[3] NUCLEOTIDE SEQUENCE [LARGE SCALE MRNA].
STRAIN=cv. Columbia;
DOI=10.1126/science.1088305;MEDLINE=22954850;PubMed=14593172 [NCBI, ExPASy, EBI, Israel, Japan]
Yamada K., Lim J., Dale J.M., Chen H., Shinn P., Palm C.J., Southwick A.M., Wu H.C., Kim C.J., Nguyen M., Pham P.K., Cheuk R.F., Karlin-Newmann G., Liu S.X., Lam B., Sakano H., Wu T., Yu G., Miranda M., , Ecker J.R.;
"Empirical analysis of transcriptional activity in the Arabidopsis genome.";
Science 302:842-846(2003).

[4] NUCLEOTIDE SEQUENCE [LARGE SCALE MRNA].
Brover V., Troukhan M., Alexandrov N., Lu Y.-P., Flavell R., Feldmann K.A.;
"Full-length cDNA from Arabidopsis thaliana.";
Submitted (MAR-2002) to the EMBL/GenBank/DDBJ databases.

[5] FUNCTION.
DOI=10.1105/tpc.9.4.509;PubMed=9144959 [NCBI, ExPASy, EBI, Israel, Japan]
Epple P., Apel K., Bohlmann H.;
"Overexpression of an endogenous thionin enhances resistance of Arabidopsis against Fusarium oxysporum.";
Plant Cell 9:509-520(1997).

[6] FUNCTION, TISSUE SPECIFICITY, AND INDUCTION.
PubMed=9628023 [NCBI, ExPASy, EBI, Israel, Japan]
Vignutelli A., Wasternack C., Apel K., Bohlmann H.;
"Systemic and local induction of an Arabidopsis thionin gene by wounding and pathogens.";
Plant J. 14:285-295(1998).

Comments

- ***FUNCTION***: Seems to function as a defense factor. Thionins are small plant proteins which are toxic to animal cells. They seem to exert their toxic effect at the level of the cell membrane. Their precise function is not known.
- ***SUBCELLULAR LOCATION***: Secreted *(Potential)*.
- ***TISSUE SPECIFICITY***: Detected in rosette leaves and at a very high level in flowers and in siliques.
- ***INDUCTION***: Highly induced in seedlings by pathogens, wounding, silver nitrate, and methyl jasmonate.
- ***SIMILARITY***: Belongs to the plant thionin (TC 1.C.44) family [view classification].

Copyright

Cross-references

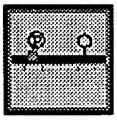
	L41244; AAC41678.1; -.	[EMBL / GenBank / DDBJ] [CoDingSequence]
EMBL	AC067754; AAG51790.1; ALT_INIT.	[EMBL / GenBank / DDBJ] [CoDingSequence]
	AY080781; AAL87264.1; -.	[EMBL / GenBank / DDBJ]

	AY086595; AAM63655.1; ALT_INIT.	[CoDingSequence] [EMBL / GenBank / DDBJ] [CoDingSequence]
PIR	C96746; C96746.	
HSSP	P32880; 1JMN. [HSSP ENTRY / PDB]	
GeneFarm	3048; 293.	
TAIR	At1g72260; Q42596.	
InterPro	IPR001010; Thionin. Graphical view of domain structure.	
Pfam	PF00321; Thionin; 1. Pfam graphical view of domain structure.	
PROSITE	PS00271; THIONIN; 1.	
ProDom	[Domain structure / List of seq. sharing at least 1 domain]	
BLOCKS	Q42596.	
ProtoNet	Q42596.	
ProtoMap	Q42596.	
PRESAGE	Q42596.	
DIP	Q42596.	
ModBase	Q42596.	
SMR	Q42596; 515B40A0DBDCC1EF.	
SWISS-2DPAGE	Get region on 2D PAGE.	
UniRef	View cluster of proteins with at least 50% / 90% identity.	

Keywords

Plant defense; **Plant toxin**; **Signal**; **Thionin**; **Toxin**.

Features

 Feature table viewer

 Feature aligner

Key	From	To	Length	Description
SIGNAL	1	24	24	*Potential.*
CHAIN	25	67	43	Thionin 2.1.
CHAIN	68	134	67	Acidic protein *(Potential).*
DISULFID	27	61		*By similarity.*
DISULFID	28	55		*By similarity.*
DISULFID	40	49		*By similarity.*
CONFLICT	7	7		I -> S (in Ref. 4).
CONFLICT	74	74		A -> G (in Ref. 4).

Sequence information

Length: **134 AA** [This is the length of the unprocessed precursor]

Molecular weight: **14351 Da** [This is the MW of the unprocessed precursor]

CRC64: **515B40A0DBDCC1EF** [This is a checksum on the sequence]

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TKSYVVPPGP PKLL
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Q42596 in FASTA format

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Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

ScanProsite, MotifScan

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NiceProt View of Swiss-Prot: P32032

[Entry info] [Name and origin] [References] [Comments] [Cross-references] [Keywords] [Features] [Sequence] [Tools]

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**THN2_WHEAT**
Primary accession number	**P32032**
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 26, July 1993
Sequence was last modified in	Release 26, July 1993
Annotations were last modified in	Release 46, February 2005

Name and origin of the protein

Protein name	**Alpha-2-purothionin [Precursor]**
Synonyms	None
Gene name	**Name: THI1.2** Synonyms: PUR-B1
From	Triticum aestivum (Wheat) [TaxID: 4565]
Taxonomy	Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Pooideae; Triticeae; Triticum.

References

[1] NUCLEOTIDE SEQUENCE.
TISSUE=Endosperm;
DOI=10.1104/pp.106.3.1221;MEDLINE=95125120;PubMed=7824649 [NCBI, ExPASy, EBI, Israel, Japan]
Castagnaro A., Marana C., Carbonero P., Garcia-Olmedo F.;
"cDNA cloning and nucleotide sequences of alpha 1 and alpha 2 thionins from hexaploid wheat endosperm.";
Plant Physiol. 106:1221-1222(1994).

Comments

- ***FUNCTION***: Thionins are small plant proteins which are toxic to animal cells. They seem to exert their toxic effect at the level of the cell membrane. Their precise function is not known.
- ***SUBCELLULAR LOCATION***: Secreted.
- ***SIMILARITY***: Belongs to the plant thionin (TC 1.C.44) family [view classification].

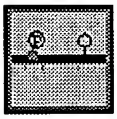
Cross-references

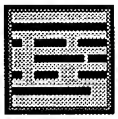
EMBL	X70665; CAA50003.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]
PIR	S31695; S31695.
HSSP	P01543; 1BHP. [HSSP ENTRY / PDB]
InterPro	IPR001010; Thionin. Graphical view of domain structure.
Pfam	PF00321; Thionin; 1. Pfam graphical view of domain structure.
PRINTS	PR00287; THIONIN.
PROSITE	PS00271; THIONIN; 1.
ProDom	[Domain structure / List of seq. sharing at least 1 domain]
BLOCKS	P32032.
ProtoNet	P32032.
ProtoMap	P32032.
PRESAGE	P32032.
DIP	P32032.
ModBase	P32032.
SMR	P32032; B4019F014E226B9F.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

Plant defense; **Plant toxin**; **Signal**; **Thionin**.

Features

 Feature table viewer

 Feature aligner

Key	From	To	Length	Description
SIGNAL	1	27	27	
CHAIN	28	72	45	Alpha-2-purothionin.
CHAIN	73	136	64	Acidic protein.
DISULFID	30	66		*By similarity.*
DISULFID	31	58		*By similarity.*
DISULFID	39	56		*By similarity.*
DISULFID	43	52		*By similarity.*

Sequence information

Length: **136 AA** [This is the length of the unprocessed precursor]

Molecular weight: **14558 Da** [This is the MW of the unprocessed precursor]

CRC64: **B4019F014E226B9F** [This is a checksum on the sequence]

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MGSKGLKGVM VCLLILGLVL EQVQVEGKSC CRTTLGRNCY NLCRSRGAQK LCSTVCRCKL

        70         80         90        100        110        120
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TSGLSCPKGF PKLALESNSD EPDTIEYCNL GCRSSVCDYM VNAAADDEEM KLYVENCGDA

      130
CVNFCNGDAG LTSLDA
```

P32032 in FASTA format

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BLAST submission on ExPASy/SIB or at NCBI (USA)

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

ScanProsite, MotifScan

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NiceProt View of Swiss-Prot: P01543

Entry information

Entry name	**THNB_WHEAT**
Primary accession number	**P01543**
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 01, July 1986
Sequence was last modified in	Release 36, July 1998
Annotations were last modified in	Release 46, February 2005

Name and origin of the protein

Protein name	**Purothionin A-I [Precursor]**
Synonym	**Beta-purothionin**
Gene name	**Name: THI1.3**
From	Triticum aestivum (Wheat) [TaxID: 4565]
Taxonomy	Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Pooideae; Triticeae; Triticum.

References

[1] NUCLEOTIDE SEQUENCE.
STRAIN=cv. Rosella;
Hughes P.A., Llewellyn D.L., Whitecross M.;
Submitted (JUN-1997) to the EMBL/GenBank/DDBJ databases.

[2] PROTEIN SEQUENCE OF 28-72.
STRAIN=cv. Manitoba 3;
MEDLINE=78026451;PubMed=914810 [NCBI, ExPASy, EBI, Israel, Japan]
Ohtani S., Okada T., Yoshizumi H., Kagamiyama H.;
"Complete primary structures of two subunits of purothionin A, a lethal protein for brewer's yeast from wheat flour.";
J. Biochem. 82:753-767(1977).

[3] PROTEIN SEQUENCE OF 28-72.
Ohtani S., Okada T., Kagamiyama H., Yoshizumi H.;
"The amino acid sequence of purothionin A, a lethal toxic protein to brewer's yeast from wheat.";

Agric. Biol. Chem. 39:2269-2270(1975).
[4] PROTEIN SEQUENCE OF 28-72.
MEDLINE=77046666;PubMed=990986 [NCBI, ExPASy, EBI, Israel, Japan]
Mak A.S., Jones B.L.;
"The amino acid sequence of wheat beta-purothionin.";
Can. J. Biochem. 54:835-842(1976).
[5] X-RAY CRYSTALLOGRAPHY (1.7 ANGSTROMS).
DOI=10.1107/S0907444995002976;PubMed=15299761 [NCBI, ExPASy, EBI, Israel, Japan]
Stec B., Rao U., Teeter M.M.;
"Refinement of purothionins reveals solute particles important for lattice formation and toxicity. Part 2: structure of beta-purothionin at 1.7-A resolution.";
Acta Crystallogr. D 51:914-924(1995).

Comments

- ***FUNCTION***: Thionins are small plant proteins which are toxic to animal cells. They seem to exert their toxic effect at the level of the cell membrane. Their precise function is not known.
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- ***SIMILARITY***: Belongs to the plant thionin (TC 1.C.44) family [view classification].

Copyright

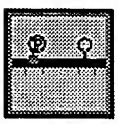
Cross-references

EMBL	AF004018; AAB71137.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]
PDB	1BHP; X-ray; @=28-71.[ExPASy / RCSB / EBI]
InterPro	IPR001010; Thionin. Graphical view of domain structure.
Pfam	PF00321; Thionin; 1. Pfam graphical view of domain structure.
PRINTS	PR00287; THIONIN.
PROSITE	PS00271; THIONIN; 1.
ProDom	[Domain structure / List of seq. sharing at least 1 domain]
BLOCKS	P01543.
ProtoNet	P01543.
ProtoMap	P01543.
PRESAGE	P01543.
DIP	P01543.
ModBase	P01543.
SMR	P01543; A855C815519EDA24.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

3D-structure; **Direct protein sequencing**; **Plant defense**; **Plant toxin**; **Signal**; **Thionin**.

Features

 Feature table viewer

 Feature aligner

Key	From	To	Length	Description
SIGNAL	1	27	27	
CHAIN	28	72	45	Purothionin A-I.
CHAIN	73	136	64	Acidic protein.
DISULFID	30	66		
DISULFID	31	58		
DISULFID	39	56		
DISULFID	43	52		
STRAND	29	31	3	
HELIX	34	43	10	
TURN	44	46	3	
HELIX	49	55	7	
TURN	56	57	2	
STRAND	58	60	3	
TURN	68	69	2	

Sequence information

Length: **136 AA** [This is the length of the unprocessed precursor]

Molecular weight: **14625 Da** [This is the MW of the unprocessed precursor]

CRC64: **A855C815519EDA24** [This is a checksum on the sequence]

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        70         80         90        100        110        120
TSGLSCPKDF PKLVLESNSD EPDTMEYCNL GCRSSLCDYI VNAAADDEEM KLYVEQCGDA

       130
CVNFCNADAG LTSLDA
```

P01543 in FASTA format

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BLAST submission on ExPASy/SIB or at NCBI (USA)

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

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Acta Crystallographica Section D

Biological Crystallography

Volume 51, Part 6 (November 1995)

research papers

Acta Cryst. (1995). D**51**, 914-924 [doi:10.1107/S0907444995002976]

Refinement of purothionins reveals solute particles important for lattice formation and toxicity. Part 2: structure of β-purothionin at 1.7 Å resolution

B. Stec, U. Rao and M. M. Teeter

Abstract: The crystal structure of β-purothionin (β-PT) has been determined at 1.7 Å resolution. β-PT and previously solved α_1-PT belong to a family of membrane-active plant toxins homologous to crambin. (β-PT crystallizes in the same space group as α_1-PT (1422) but with the *c* axis 3 Å longer than (α_1-PT. The unit-cell dimensions of β-PT crystals are $a = b = 53.94$ and $c = 72.75$ Å. Two data sets were collected on a multiwire area detector, each with R_{sym} around 6.0%, and were merged to get a single data set at 1.7 Å, ($R_{merge} = 9.6\%$). The X-ray structure of α_1-PT was used to build a starting model for β-PT. The β-PT model was refined using the program *PROLSQ* from 10 to 1.7 Å resolution to an *R*-factor of 19.8% with very good geometry. The final structure contains 439 atoms including 337 protein atoms, 77 waters, two acetates, two glycerols and one phosphate. The high-resolution structure of the β-PT agreed well with that of the lower resolution α_1-PT structure only after the latter was extensively rerefined. Both refinements revealed phosphate and glycerol molecules which are important in lattice formation. The binding of phosphate and glycerol molecules to purothionins (PT) was confirmed by NMR and was implicated in the biological activity of toxins. Modeling of phospholipid binding to PT based on glycerol and phosphate-binding site could shed light on the lytic toxicity of this protein-toxin family. Although the structures of (α_1-PT and β-PT preserve the overall fold of crambin, they exhibit key differences that are directly relevant to the toxicity of thionins.

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NCBI BLAST program reference [PMID:9254694]:
Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402(1997).

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Query: 136 AA
Date run: 2005-02-24 18:07:40 UTC+0100 on sib-gm1.unil.ch
Program: NCBI BLASTP 1.5.4-Paracel [2003-06-05]
Database: EXPASY/UniProt
1,794,555 sequences; 574,459,479 total letters
UniProt Release 4.1 consists of: Swiss-Prot Release 46.1 of 15-Feb-2005: 170140 ent
TrEMBL Release 29.1 of 15-Feb-2005: 1614107 entries

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List of potentially matching sequences

Send selected sequences to Clustal W (multiple alignment) Submit Query
Select up to...

☐ Include query sequence

	Db	AC	Description	Score	E-value
☐	sp	P32032	THN2_WHEAT Alpha-2-purothionin precursor [THI1.2] [Tri...	288	1e-77
☐	tr	Q43205	_WHEAT Alpha-1 purothionin [Triticum aestivum (Wheat)]	285	8e-77
☐	sp	P01543	THNB_WHEAT Purothionin A-I precursor (Beta-purothionin...	268	1e-71
☐	tr	Q9T0P1	_WHEAT Alpha purothionin precursor [Pur-B1] [Triticum a...	268	1e-71
☐	tr	Q9T0P2	_WHEAT Beta purothionin precursor [Pur-A1] [Triticum ae...	267	3e-71
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☐	tr	Q9ZNY5	_SECCE Purothionin precursor [Pur-RL] [Secale cereale (...	257	3e-68
☐	sp	P01544	THN1_WHEAT Alpha-1-purothionin precursor (Purothionin ...	251	2e-66

☐	sp	P01545	THNA_HORVU Alpha-hordothionin precursor (Purothionin I...	243 5e-64
☐	tr	Q9S6Y2	_WHEAT Alpha purothionin (Fragment) [Pur-B1] [Triticum ...	181 2e-45
☐	tr	Q9S9D7	_HORVU Thionin [Hordeum vulgare (Barley)]	155 2e-37
☐	sp	Q42838	THN7_HORVU Thionin BTH7 precursor [Hordeum vulgare (Ba...	154 3e-37
☐	tr	Q8LT03	_AVESA Leaf thionin Asthi1 [Asthi1] [Avena sativa (Oat)]	154 4e-37
☐	sp	P08772	THN3_HORVU Leaf-specific thionin DB4 precursor [THI1.3...	153 6e-37
☐	sp	Q8H0Q5	THNX_HORVU Probable leaf thionin precursor [Hordeum vu...	152 1e-36
☐	sp	P09617	THN5_HORVU Leaf-specific thionin precursor (Clones PKG...	152 1e-36
☐	sp	P09618	THN6_HORVU Leaf-specific thionin BTH6 precursor [Horde...	149 9e-36
☐	tr	Q8LT02	_AVESA Leaf thionin Asthi2 [Asthi2] [Avena sativa (Oat)]	145 1e-34
☐	tr	Q8LSZ9	_AVESA Thionin Asthi5 [Asthi5] [Avena sativa (Oat)]	138 2e-32
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☐	tr	Q41585	_WHEAT Type V Thionin precursor [TthV2] [Triticum aesti...	134 4e-31
☐	sp	Q05806	THN5_WHEAT Type V thionin precursor [TTHV] [Triticum a...	130 6e-30
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☐	tr	Q38770	_AEGTA Type V Thionin precursor [AthV1] [Aegilops tausc...	129 8e-30
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☐	tr	Q5Z5V1	_ORYSA Putative thionin Osthi1 [OSJNBa0020P04.23] [Oryz...	106 9e-23
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☐	tr	Q5Z4S4	_ORYSA Putative thionin Osthi1 [P0597A07.39] [Oryza sat...	102 1e-21
☐	tr	Q8LT04	_ORYSA Thionin Osthi1 [Osthi1] [Oryza sativa (japonica ...	102 1e-21
☐	tr	Q5Z4K0	_ORYSA Thionin Osthi1 [OSJNBa0022O06.36] [Oryza sativa ...	101 2e-21
☐	tr	Q9S975	_CRAAB Crambin PRECURSOR=THIONIN variant THI2CA11 (Frag...	90 7e-18
☐	tr	Q43225	_TULGE Thionin class 1 precursor [Thi1-2] [Tulipa gesne...	90 7e-18
☐	sp	Q9SBK8	THN_BRARP Thionin precursor [THI2] [Brassica rapa subs...	89 1e-17
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☐	tr	Q9S976	_CRAAB Crambin PRECURSOR=THIONIN variant THI2CA10 (Frag...	86 1e-16
☐	tr	Q43226	_TULGE Thionin class 1 precursor [Thi1-3] [Tulipa gesne...	86 1e-16
☐	tr	Q9S981	_CRAAB Crambin PRECURSOR=THIONIN variant THI2CA2 (Fragm...	85 2e-16
☐	tr	Q9S974	_CRAAB Crambin PRECURSOR=THIONIN variant THI2CA12 (Frag...	84 4e-16
☐	tr	Q9S9A0	_VISAL Thionin [Viscum album (European mistletoe)]	83 8e-16
☐	sp	Q42597	THN22_ARATH Thionin 2.2 precursor [THI2.2] [Arabidopsi...	83 1e-15
☐	tr	Q43227	_TULGE Thionin class 1 precursor (Fragment) [Thi1-4] [T...	82 2e-15
☐	tr	Q9S980	_CRAAB Crambin PRECURSOR=THIONIN variant THI2CA3 (Fragm...	81 4e-15
☐	tr	Q9S977	_CRAAB Crambin PRECURSOR=THIONIN variant THI2CA9 (Fragm...	80 5e-15
☐	sp	Q42596	THN21_ARATH Thionin 2.1 precursor [THI2.1] [Arabidopsi...	79 1e-14
☐	sp	P01538	THN3_VISAL Viscotoxin A3 precursor [THI2.1] [Viscum al...	77 4e-14
☐	tr	Q9S9A2	_VISAL Viscotoxin A [Viscum album (European mistletoe)]	77 4e-14
☐	tr	Q9S9A1	_VISAL Thionin [Viscum album (European mistletoe)]	74 6e-13
☐	tr	Q9S979	_CRAAB Crambin PRECURSOR=THIONIN variant THI2CA5 (Fragm...	73 8e-13
☐	sp	P08943	THNB_VISAL Viscotoxin B precursor (Fragment) [THI2.2] ...	72 1e-12
☐	sp	Q8VZK8	THN23_ARATH Probable thionin 2.3 precursor [At2g15010]...	72 2e-12
☐	tr	Q41609	_TULGE Thionin class 4 precursor [Thi4-1] [Tulipa gesne...	72 2e-12
☐	tr	Q5Z554	_ORYSA Putative thionin Osthi1 [OSJNBa0085C03.20] [Oryz...	71 3e-12

☐	sp Q9C8D6	THN24_ARATH Probable thionin 2.4 precursor [At1g66100]...	65	2e-10
☐	tr Q5Z551	_ORYSA Putative thionin Osthi1 [OSJNBa0085C03.23] [Oryz...	61	3e-09
☐	sp P07504	THN_PYRPU Thionin [THI1] [Pyrularia pubera (Rabbitwood...	61	4e-09
☐	sp P60057	THND_HELPU Hellethionin D [Helleborus purpurascens (Pu...	52	2e-06
☐	sp P83554	THNC_VISAL Viscotoxin C1 [Viscum album (European mistl...	51	4e-06
☐	sp P01541	THN_DENCL Denclatoxin B [Dendrophthora clavata (Columb...	50	6e-06
☐	sp P59358	THNB_PHOLI Ligatoxin B [Phoradendron liga (Argentine m...	48	4e-05
☐	sp P01539	THN_PHOTO Phoratoxin [Phoradendron tomentosum (Califor...	47	6e-05
☐	sp P32880	THN2_VISAL Viscotoxin A2 [THI2.3] [Viscum album (Europ...	47	6e-05
☐	tr Q5Z553	_ORYSA Hypothetical protein OSJNBa0085C03.21 [OSJNBa008...	47	8e-05
☐	sp P01540	THNA_PHOLI Ligatoxin A [Phoradendron liga (Argentine m...	45	2e-04
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☐	sp P01537	THN1_VISAL Viscotoxin 1-PS [THI2.4] [Viscum album (Eur...	44	4e-04
☐	tr Q5Z557	_ORYSA Hypothetical protein OSJNBa0085C03.15 [OSJNBa008...	44	4e-04
☐	tr Q9S978	_CRAAB Crambin PRECURSOR=THIONIN variant THI2CA7 (Fragm...	42	0.002
☐	sp O88745	SCRG1_MOUSE Scrapie-responsive protein 1 precursor (Sc...	35	0.19
☐	sp P01542	CRAM_CRAAB Crambin [THI2] [Crambe abyssinica (Abyssini...	35	0.25
☐	sp Q9Z0K6	SCRG1_RAT Scrapie-responsive protein 1 precursor (ScRG...	34	0.43
☐	tr Q9LN81	_ARATH T12C24.19 [Arabidopsis thaliana (Mouse-ear cress)]	34	0.57
☐	tr Q7Q1J5	_ANOGA ENSANGP00000014375 (Fragment) [ENSANGG0000001188...	33	0.74
☐	tr Q8W2K6	_9SOLN Proteinase inhibitor IIa [PIN2a] [Solanum americ...	33	0.96
☐	tr Q8MY77	_BRABE Bb-cadherin [BbCad] [Branchiostoma belcheri (Amp...	32	1.6
☐	tr Q8BKK7	_MOUSE Mus musculus 12 days embryo spinal ganglion cDNA...	32	2.1
☐	tr Q95V69	_TETTH Cell surface immobilization antigen SerH6 [SerH]...	32	2.8
☐	tr Q60XC0	_CAEBR Hypothetical protein CBG18730 (Fragment) [CBG187...	32	2.8
☐	sp P04881	NCAP_VSVJO Nucleocapsid protein (Nucleoprotein) [N] [V...	31	3.7
☐	tr Q89779	_9RHAB (strain 09/82-HD-B) nucleoprotein ((strain 06/85...	31	3.7
☐	tr Q89594	_9RHAB (strain 10/85-HD-B1) nucleoprotein ((strain 12/8...	31	3.7
☐	tr Q89520	_9RHAB (strain ../49-UT-B1) nucleoprotein ((strain ../5...	31	3.7
☐	tr Q89040	_9RHAB (strain ../60-PN-B) nucleoprotein [Vesicular sto...	31	3.7
☐	tr Q89039	_9RHAB (strain 01/85-PN-B1) nucleoprotein [Vesicular st...	31	3.7
☐	tr Q89038	_9RHAB (strain 07/83-NC-P) nucleoprotein [Vesicular sto...	31	3.7
☐	tr Q89037	_9RHAB (strain 10/82-CR-B) nucleoprotein [Vesicular sto...	31	3.7
☐	tr Q89036	_9RHAB (strain 11/84-HD-B1) nucleoprotein [Vesicular st...	31	3.7
☐	tr Q89035	_9RHAB (strain 10/84-GM-P) nucleoprotein [Vesicular sto...	31	3.7
☐	tr Q89034	_9RHAB (strain 07/84-OA-B) nucleoprotein [Vesicular sto...	31	3.7
☐	tr Q6TXD9	_9RHAB Nucleocapsid protein [Vesicular stomatitis virus]	31	3.7
☐	tr Q9CA14	_ARATH Putative phorbol ester / diacylglycerol binding ...	31	3.7
☐	tr Q620M9	_CAEBR Hypothetical protein CBG02727 [CBG02727] [Caenor...	31	3.7
☐	tr Q84BD4	_MYXXA CHP [Myxococcus xanthus]	31	4.8
☐	tr Q69JY6	_ORYSA Putative thaumatin-like protein [P0569E11.21] [O...	31	4.8
☐	tr Q6PTE0	_MONBE Methionine adenosyltransferase (Fragment) [Monos...	31	4.8
☐	tr Q6PVY8	_ORYSA Zinc finger protein [Tranpr] [Oryza sativa (japo...	30	6.3
☐	tr Q6AVT3	_ORYSA Hypothetical protein OSJNBa0027J18.18 [OSJNBa002...	30	6.3
☐	tr Q95RQ1	_DROME LD16414p (CG7447-PA, isoform A) (Cg7447-pb, isof...	30	6.3

☐ tr Q7PM75 _ANOGA ENSANGP00000010141 (Fragment) [ENSANGG0000000765... 30 6.3
☐ sp P79847 LYSC_PYGNE Lysozyme C precursor (EC 3.2.1.17) (1,4-bet... 30 8.2

Graphical overview of the alignments

Click here to resubmit your query after masking regions matching PROSITE profiles or Pfam HMMs
(? Help) (use ScanProsite for more details about PROSITE matches)

Profile hits
Pfam hits Thionin


Matches on query sequence
Mat
Submission
1
50
100
1
THN2_WHEAT
Q43205
THNB_WHEAT
Q9T0P1
Q9T0P2
THNB_HORVU
Q9ZNY5
THN1_WHEAT
THNA_HORVU
Q9S6Y2
Q9S9D7
THN7_HORVU
Q8LT03
THN3_HORVU
THNX_HORVU
THN5_HORVU
THN6_HORVU
Q8LT02
Q8LSZ9
Q8LT00
Q41585
THN5_WHEAT
Q8LT01
Q38770
Q5Z540
Q5Z4W6
Q5Z5V1
Q5Z434
Q5Z4S4
Q8LT04
Q5Z4K0
Q9S975
Q43225
THN_BRARP
Q43224
Q9S976
Q43226
Q9S981
Q9S974
Q9S9A0
THN22_ARATH
Q43227
Q9S980
Q9S977
THN21_ARATH
THN3_VISAL
Q9S9A2
Q9S9A1
Q9S979
THNB_VISAL
THN23_ARATH
Q41609
Q5Z554
THN24_ARATH
Q5Z551
THN_PYRPU
THND_HELPU
THNC_VISAL
THN_DENCL
THNB_PHOLI
THN_PHOTO
THN2_VISAL
Q5Z553
THNA_PHOLI
Q5Z4S0
THN1_VISAL
Q5Z557
Q9S978
SCRG1_MOUSE
CRAM_CRAAB
SCRG1_RAT
Q9LN81
Q7Q1J5
Q8W2K6
Q8MY77
Q8BKK7
Q95V69
Q60XC0
NCAP_VSVJO
Q89779
Q89594
Q89520
Q89040
Q89039
Q89038
Q89037

Alignments

sp P32032 THN2_WHEAT **Alpha-2-purothionin precursor [THI1.2] [Triticum aestivum (Wheat)]** 136 AA align

Score = 288 bits (737), Expect = 1e-77
Identities = 136/136 (100%), Positives = 136/136 (100%)

```
Query: 1   MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60
           MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL
Sbjct: 1   MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
           TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA
Sbjct: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120

Query: 121 CVNFCNGDAGLTSLDA 136
           CVNFCNGDAGLTSLDA
Sbjct: 121 CVNFCNGDAGLTSLDA 136
```

tr Q43205 Q43205_WHEAT **Alpha-1 purothionin [Triticum aestivum (Wheat)]** 136 AA align

Score = 285 bits (730), Expect = 8e-77
Identities = 135/136 (99%), Positives = 135/136 (99%)

```
Query: 1   MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60
           MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL
Sbjct: 1   MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
           TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA
Sbjct: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120

Query: 121 CVNFCNGDAGLTSLDA 136
           CVNFCNGDAGLTS DA
Sbjct: 121 CVNFCNGDAGLTSPDA 136
```

sp P01543 THNB_WHEAT **Purothionin A-I precursor (Beta-purothionin) [THI1.3] [Triticum aestivum (Wheat)]** 136 AA align

Score = 268 bits (686), Expect = 1e-71
Identities = 124/136 (91%), Positives = 131/136 (96%)

```
Query: 1   MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60
           MGSKGLKGVMVCLLILGLVLEQVQVEGKSCC++TLGRNCYNLCR+RGAQKLC+ VCRCKL
Sbjct: 1   MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCKSTLGRNCYNLCRARGAQKLCANVCRCKL 60

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
```

```
           TSGLSCPK FPKL LESNSDEPDT+EYCNLGCRSS+CDY+VNAAADDEEMKLYVE CGDA
Sbjct: 61  TSGLSCPKDFPKLVLESNSDEPDTMEYCNLGCRSSLCDYIVNAAADDEEMKLYVEQCGDA 120

Query: 121 CVNFCNGDAGLTSLDA 136
           CVNFCN DAGLTSLDA
Sbjct: 121 CVNFCNADAGLTSLDA 136
```

tr Q9T0P1 Q9T0P1_WHEAT **Alpha purothionin precursor [Pur-B1] [Triticum aestivum (Wheat)]** 137 AA align

```
 Score =  268 bits (686), Expect = 1e-71
 Identities = 124/136 (91%), Positives = 132/136 (96%)

Query: 1   MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60
           MGSKG KGV+VCLLILGLVLEQ+QVEGKSCCR+TLGRNCYNLCR+RGAQKLC+ VCRCK+
Sbjct: 1   MGSKGFKGVIVCLLILGLVLEQLQVEGKSCCRSTLGRNCYNLCRARGAQKLCAGVCRCKI 60

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
           +SGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENC DA
Sbjct: 61  SSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCADA 120

Query: 121 CVNFCNGDAGLTSLDA 136
           CV+FCNGDAGL SLDA
Sbjct: 121 CVSFCNGDAGLPSLDA 136
```

tr Q9T0P2 Q9T0P2_WHEAT **Beta purothionin precursor [Pur-A1] [Triticum aestivum (Wheat)]** 136 AA align

```
 Score =  267 bits (682), Expect = 3e-71
 Identities = 124/136 (91%), Positives = 130/136 (95%)

Query: 1   MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60
           MGSKGLKGVMVCLLILGLVLEQVQVEGKSCC++TLGRNCYNLCR+RGAQKLC+ VCRCKL
Sbjct: 1   MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCKSTLGRNCYNLCRARGAQKLCANVCRCKL 60

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
           TSGLSCPK FPKL LESNSDEPDT+EYCNL CRSS+CDYMVNAAADDEEMKLYVE CGDA
Sbjct: 61  TSGLSCPKDFPKLVLESNSDEPDTMEYCNLECRSSLCDYMVNAAADDEEMKLYVEQCGDA 120

Query: 121 CVNFCNGDAGLTSLDA 136
           CVNFCN DAGLTSLDA
Sbjct: 121 CVNFCNADAGLTSLDA 136
```

sp P21742 THNB_HORVU **Beta-hordothionin precursor [THI1.2] [Hordeum vulgare (Barley)]** 136 AA align

```
 Score =  257 bits (657), Expect = 2e-68
```

```
 Identities = 119/136 (87%), Positives = 125/136 (91%)

Query: 1   MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60
           MGSKGLKGVMVCLLILGLVLE VQVEGKSCCR+TLGRNCYNLCR RGAQKLC+  CRCKL
Sbjct: 1   MGSKGLKGVMVCLLILGLVLEHVQVEGKSCCRSTLGRNCYNLCRVRGAQKLCANACRCKL 60

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
           TSGL CP  FPKLAL SNSDEPDTI+YCNLGCR+S+CDYMVNAAADDEEMKLYVE+C DA
Sbjct: 61  TSGLKCPSSFPKLALVSNSDEPDTIDYCNLGCRASMCDYMVNAAADDEEMKLYVEHCSDA 120

Query: 121 CVNFCNGDAGLTSLDA 136
           CVNFCNGD GLTSL A
Sbjct: 121 CVNFCNGDVGLTSLTA 136
```

tr Q9ZNY5 **Purothionin precursor [Pur-RL] [Secale cereale (Rye)]** 136 AA
Q9ZNY5_SECCE

align

```
 Score =  257 bits (656), Expect = 3e-68
 Identities = 119/136 (87%), Positives = 127/136 (92%)

Query: 1   MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60
           MGSKGLKGVMVCLLILGLVLEQVQVEGKSCC++TLGRNCYNLCR+RGAQKLC+  CRCKL
Sbjct: 1   MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCKSTLGRNCYNLCRTRGAQKLCANFCRCKL 60

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
            S  SCPK FPKLALESNSDEPDT+EYCNLGCRSSVCDYMV+AAAD+EEMKLYVE CGDA
Sbjct: 61  ISSTSCPKEFPKLALESNSDEPDTVEYCNLGCRSSVCDYMVSAAADEEEMKLYVERCGDA 120

Query: 121 CVNFCNGDAGLTSLDA 136
           CV+FCNGDAGL SL A
Sbjct: 121 CVSFCNGDAGLPSLSA 136
```

sp P01544 **Alpha-1-purothionin precursor (Purothionin A-II)** 126
THN1_WHEAT **(Fragment)** AA
[THI1.1] [Triticum aestivum (Wheat)] align

```
 Score =  251 bits (640), Expect = 2e-66
 Identities = 115/125 (92%), Positives = 122/125 (97%)

Query: 12  CLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSGLSCPKGFP 71
           CLLILGLVLEQ+QVEGKSCCR+TLGRNCYNLCR+RGAQKLC+ VCRCK++SGLSCPKGFP
Sbjct: 1   CLLILGLVLEQLQVEGKSCCRSTLGRNCYNLCRARGAQKLCAGVCRCKISSGLSCPKGFP 60

Query: 72  KLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVNFCNGDAGL 131
           KLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENC DACV+FCNGDAGL
Sbjct: 61  KLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCADACVSFCNGDAGL 120

Query: 132 TSLDA 136
            SLDA
Sbjct: 121 PSLDA 125
```

tr Q9S6Y2 Q9S6Y2_WHEAT **Alpha purothionin (Fragment) [Pur-B1] [Triticum aestivum (Wheat)]** 90 AA align

Score = 181 bits (460), Expect = 2e-45
Identities = 83/89 (93%), Positives = 86/89 (96%)

```
Query: 48  AQKLCSTVCRCKLTSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD 107
           AQKLC+ VCRCK+ SGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD
Sbjct: 1   AQKLCAGVCRCKIASGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD 60

Query: 108 EEMKLYVENCGDACVNFCNGDAGLTSLDA 136
           EEMKLYVENCGDACV+FCNGDAGL SLDA
Sbjct: 61  EEMKLYVENCGDACVSFCNGDAGLPSLDA 89
```

tr Q9S9D7 Q9S9D7_HORVU **Thionin [Hordeum vulgare (Barley)]** 137 AA align

Score = 155 bits (391), Expect = 2e-37
Identities = 73/135 (54%), Positives = 94/135 (69%), Gaps = 2/135 (1%)

```
Query: 3   SKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLT 61
           SK +K V++C+LILGLVLEQVQVEGKSCC+ TL RNCYN CR + G++ +C+  CRCK+
Sbjct: 4   SKSIKSVVICVLILGLVLEQVQVEGKSCCKDTLARNCYNTCRFAGGSRPVCAGACRCKII 63

Query: 62  SGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDAC 121
           SG  CP  +PKL L   S EPD  +YC +GCR+SVCD M N     +EMK  +  C +AC
Sbjct: 64  SGPKCPSDYPKLNLLPESGEPDVTQYCTIGCRNSVCDNMDNVFR-GQEMKFDMGLCSNAC 122

Query: 122 VNFCNGDAGLTSLDA 136
             FCN  A + S++A
Sbjct: 123 ARFCNDGAVIQSVEA 137
```

sp Q42838 THN7_HORVU **Thionin BTH7 precursor [Hordeum vulgare (Barley)]** 137 AA align

Score = 154 bits (389), Expect = 3e-37
Identities = 71/135 (52%), Positives = 96/135 (70%), Gaps = 2/135 (1%)

```
Query: 3   SKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLT 61
           +K +K V++C+LILGLVLEQVQVEGKSCC+ T GRNCYN CR + G++ +C+T C CK+
Sbjct: 4   NKSIKSVVICVLILGLVLEQVQVEGKSCCKNTTGRNCYNACRFAGGSRPVCATACGCKII 63

Query: 62  SGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDAC 121
           SG +CP+ +PKL L   S EP+  EYC +GCR+SVCD M N +   +EMK  +  C +AC
Sbjct: 64  SGPTCPRDYPKLNLLPESGEPNVTEYCTIGCRTSVCDNMDNVSR-GQEMKFDMGLCSNAC 122

Query: 122 VNFCNGDAGLTSLDA 136
             FCN    + S++A
Sbjct: 123 ARFCNDGEVIQSVEA 137
```

sp P08772 THN3_HORVU **Leaf-specific thionin DB4 precursor [THI1.3] [Hordeum vulgare (Barley)]** 137 AA align

Score = 153 bits (386), Expect = 6e-37
Identities = 72/135 (53%), Positives = 93/135 (68%), Gaps = 2/135 (1%)

```
Query: 3   SKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLT 61
           SK +K V++C+LILGLVLEQVQVEGKSCC+ TL RNCYN C  + G++ +C+  CRCK+
Sbjct: 4   SKSIKSVVICVLILGLVLEQVQVEGKSCCKDTLARNCYNTCHFAGGSRPVCAGACRCKII 63

Query: 62  SGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDAC 121
           SG  CP  +PKL L   S EPD  +YC +GCR+SVCD M N     +EMK  +  C +AC
Sbjct: 64  SGPKCPSDYPKLNLLPESGEPDVTQYCTIGCRNSVCDNMDNVFR-GQEMKFDMGLCSNAC 122

Query: 122 VNFCNGDAGLTSLDA 136
             FCN  A + S++A
Sbjct: 123 ARFCNDGAVIQSVEA 137
```

sp Q8H0Q5 THNX_HORVU **Probable leaf thionin precursor [Hordeum vulgare (Barley)]** 137 AA align

Score = 152 bits (383), Expect = 1e-36
Identities = 70/135 (51%), Positives = 95/135 (69%), Gaps = 2/135 (1%)

```
Query: 3   SKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLT 61
           +K +K V++C+LILGLVLEQVQVEGKSCC+ T GRNCYN C  + G++ +C+T C CK+
Sbjct: 4   NKSIKSVVICVLILGLVLEQVQVEGKSCCKNTTGRNCYNACHFAGGSRPVCATACGCKII 63

Query: 62  SGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDAC 121
           SG +CP+ +PKL L   S EP+  EYC +GCR+SVCD M N +   +EMK  +  C +AC
Sbjct: 64  SGPTCPRDYPKLNLLPESGEPNATEYCTIGCRTSVCDNMDNVSR-GQEMKFDMGLCSNAC 122

Query: 122 VNFCNGDAGLTSLDA 136
             FCN    + S++A
Sbjct: 123 ARFCNDGDVIQSVEA 137
```

sp P09617 THN5_HORVU **Leaf-specific thionin precursor (Clones PKG1348, PKG1940, PKG2872 and DG3) [THI1.5] [Hordeum vulgare (Barley)]** 137 AA align

Score = 152 bits (383), Expect = 1e-36
Identities = 70/135 (51%), Positives = 95/135 (69%), Gaps = 2/135 (1%)

```
Query: 3   SKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLT 61
           +K +K V++C+LILGLVLEQVQVE KSCC+ T GRNCYN CR + G++ +C+T C CK+
Sbjct: 4   NKSIKSVVICVLILGLVLEQVQVEAKSCCKNTTGRNCYNACRFAGGSRPVCATACGCKII 63

Query: 62  SGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDAC 121
           SG +CP+ +PKL L   S EP+  EYC +GCR+SVCD M N +   +EMK  +  C +AC
Sbjct: 64  SGPTCPRDYPKLNLLPESGEPNATEYCTIGCRTSVCDNMDNVSR-GQEMKFDMGLCSNAC 122
```

```
Query: 122 VNFCNGDAGLTSLDA 136
           FCN    + S++A
Sbjct: 123 ARFCNDGEVIQSVEA 137
```

sp P09618 THN6_HORVU **Leaf-specific thionin BTH6 precursor [Hordeum vulgare (Barley)]** 137 AA align

```
 Score =  149 bits (376), Expect = 9e-36
 Identities = 71/135 (52%), Positives = 92/135 (67%), Gaps = 2/135 (1%)

Query: 3   SKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLT 61
           SK +K V++C+LIL LVLEQVQVEGKSCC+ TL RNCYN CR + G++ +C+  CRCK+
Sbjct: 4   SKSIKSVVICVLILVLVLEQVQVEGKSCCKDTLARNCYNTCRFAGGSRPVCAGACRCKII 63

Query: 62  SGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDAC 121
           SG  CP  +PKL L   S EPD  +YC +GC +SVCD M N      +EMK  +  C +AC
Sbjct: 64  SGPKCPSDYPKLNLLPESGEPDVTQYCTIGCTNSVCDNMDNVFR-GQEMKFDMGLCSNAC 122

Query: 122 VNFCNGDAGLTSLDA 136
           FCN  A + S++A
Sbjct: 123 ARFCNDGAVIQSVEA 137
```

tr Q8LT02 Q8LT02_AVESA **Leaf thionin Asthi2 [Asthi2] [Avena sativa (Oat)]** 137 AA align

```
 Score =  145 bits (367), Expect = 1e-34
 Identities = 71/128 (55%), Positives = 93/128 (72%), Gaps = 3/128 (2%)

Query: 1   MGS-KGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRC 58
           MGS KGLK V++C+L+LG+VLE VQVEGKSCC+ T  RNCYN+CR  G  + +C+T CRC
Sbjct: 1   MGSIKGLKSVVICVLVLGIVLEHVQVEGKSCCKDTTARNCYNVCRIPGTPRPVCATTCRC 60

Query: 59  KLTSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCG 118
           K+ SG  CPK +PKL  + ++  P+ IE+CN+GC SSV D M N A + EE ++ VE CG
Sbjct: 61  KIISGNKCPKDYPKLHGDLDAGTPNAIEFCNMGCMSSVRDNM-NNAYNIEEKEIDVELCG 119

Query: 119 DACVNFCN 126
            A  +FCN
Sbjct: 120 TAYASFCN 127
```

tr Q8LSZ9 Q8LSZ9_AVESA **Thionin Asthi5 [Asthi5] [Avena sativa (Oat)]** 136 AA align

```
 Score =  138 bits (348), Expect = 2e-32
 Identities = 69/135 (51%), Positives = 89/135 (65%), Gaps = 3/135 (2%)

Query: 3   SKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLT 61
           +KGLK V++C+L+LGLVL QVQVEGKSCC +T  RNCYN+CR  G  + + C+++C+ CK+
```

```
Sbjct: 4   TKGLKCVVLCVLVLGLVLGQVQVEGKSCCPSTSARNCYNVCRLTGTSRPRCASLCGCKIV 63

Query: 62  SGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDAC 121
            G +CP G+ KL L   S EPD  EYC +GC +SVCD M N      +EMK+ +  C   C
Sbjct: 64  DG-TCPDGYSKLHLLLESGEPDVTEYCTIGCMTSVCDNMDN-VIHGQEMKIDMLLCNKEC 121

Query: 122 VNFCNGDAGLTSLDA 136
           V FCN  A + S  A
Sbjct: 122 VRFCNKGAVIPSFQA 136
```

tr Q8LT00 Q8LT00_AVESA **Thionin Asthi4 [Asthi4] [Avena sativa (Oat)]** 142 AA align

```
 Score =  134 bits (338), Expect = 2e-31
 Identities = 67/139 (48%), Positives = 88/139 (63%), Gaps = 5/139 (3%)

Query: 3   SKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLT 61
           SKG+K  ++C L+LGLVLEQVQVEGKSCC++T   NCYN+CR  GA + +C+  C CKL
Sbjct: 4   SKGIKSAVICFLMLGLVLEQVQVEGKSCCKSTTAINCYNVCRLAGAPRPVCAGPCGCKLL 63

Query: 62  SGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNA----AADDEEMKLYVENC 117
              +CP  +PK  L S   E D  EYC +GC +SVCD + NA        +EM + ++ C
Sbjct: 64  DVTTCPSDWPKQHLLSEYGEADAAEYCTIGCMTSVCDNIGNAMFAPIVRGQEMNIDMQVC 123

Query: 118 GDACVNFCNGDAGLTSLDA 136
            +ACV FCN  A   S+ A
Sbjct: 124 NNACVRFCNKGAVNPSVGA 142
```

tr Q41585 Q41585_WHEAT **Type V Thionin precursor [TthV2] [Triticum aestivum (Wheat)]** 130 AA align

```
 Score =  134 bits (336), Expect = 4e-31
 Identities = 68/135 (50%), Positives = 87/135 (64%), Gaps = 10/135 (7%)

Query: 2   GSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKL-CSTVCRCKL 60
           G KGL+  +VCLL+LGLVLEQVQVEG  C        C+N C    +    C+  C C+L
Sbjct: 4   GKKGLESAIVCLLVLGLVLEQVQVEGVDCGANPFKVACFNSCLLGPSTVFQCADFCACRL 63

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
            +GL+  +         +SDEP+ IEYC+LGCRSSVCD +++ A + EEMKLYV+ CGDA
Sbjct: 64  PAGLASLR---------SSDEPNAIEYCSLGCRSSVCDNVISTADNTEEMKLYVKRCGDA 114

Query: 121 CVNFCNGDAGLTSLD 135
           C +FC GD  L SLD
Sbjct: 115 CDSFCKGDTLLASLD 129
```

sp Q05806 THN5_WHEAT **Type V thionin precursor [TTHV] [Triticum aestivum (Wheat)]** 131 AA align

```
 Score =  130 bits (326), Expect = 6e-30
 Identities = 69/136 (50%), Positives = 86/136 (62%), Gaps = 11/136 (8%)

Query: 2   GSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKL-CSTVCRCKL 60
           G KGL+  +VCLL+LGLVLEQVQVEG  C        C+N C    +    C+  C C+L
Sbjct: 4   GQKGLESAIVCLLVLGLVLEQVQVEGVDCGANPFKVACFNSCLLGPSTVFQCADFCACRL 63

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD-EEMKLYVENCGD 119
            +GL+  +         +SDEP+ IEYC+LGCRSSVCD M+N A +  EEMKLYV+ CG
Sbjct: 64  PAGLASVR---------SSDEPNAIEYCSLGCRSSVCDNMINTADNSTEEMKLYVKRCGV 114

Query: 120 ACVNFCNGDAGLTSLD 135
           AC +FC GD  L SLD
Sbjct: 115 ACDSFCKGDTLLASLD 130
```

tr Q8LT01 Q8LT01_AVESA **Leaf thionin Asthi3 [Asthi3] [Avena sativa (Oat)]** 137 AA align

```
 Score =  129 bits (325), Expect = 8e-30
 Identities = 64/138 (46%), Positives = 89/138 (64%), Gaps = 3/138 (2%)

Query: 1   MGS-KGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRC 58
           MGS KGL+ +++C+LI+G+VLEQVQVEG +CC+  + RNCYN+CR  G    +C+ +CRC
Sbjct: 1   MGSIKGLRSLIMCVLIVGIVLEQVQVEGNTCCKDDIARNCYNVCRIPGTPTFICANMCRC 60

Query: 59  KLTSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCG 118
            +T    CP  +PKL  + ++  P+ IE+CN+GC SS+C  M N A   EE +   E C
Sbjct: 61  IITRRNECPNDYPKLQSDLDAGTPNAIEFCNMGCMSSICGNMKN-AYPGEEKENDKEFCS 119

Query: 119 DACVNFCNGDAGLTSLDA 136
            AC  FCN     TS+ A
Sbjct: 120 IACARFCNKITVSTSVAA 137
```

tr Q38770 Q38770_AEGTA **Type V Thionin precursor [AthV1] [Aegilops tauschii (Tausch's goatgrass) (Aegilops squarrosa)]** 131 AA align

```
 Score =  129 bits (325), Expect = 8e-30
 Identities = 69/136 (50%), Positives = 86/136 (62%), Gaps = 11/136 (8%)

Query: 2   GSKGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKL-CSTVCRCKL 60
           G KGL+  +VCLL+LGLVLEQVQVEG  C        C+N C    +    C+  C C+L
Sbjct: 4   GQKGLESAIVCLLVLGLVLEQVQVEGVDCGANPFKVACFNSCLLGPSTVFQCADFCACRL 63

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD-EEMKLYVENCGD 119
            +GL+  +         +SDEP+ IEYC+LGCRSSVCD M+N A +  EEMKLYV+ CG
Sbjct: 64  PAGLASVR---------SSDEPNAIEYCSLGCRSSVCDNMINRADNSTEEMKLYVKRCGV 114

Query: 120 ACVNFCNGDAGLTSLD 135
           AC +FC GD  L SLD
Sbjct: 115 ACDSFCKGDTLLASLD 130
```

tr Q5Z4W6 Q5Z4W6_ORYSA **Putative thionin Osthi1 [OSJNBb0071G09.18] [Oryza sativa (japonica cultivar-group)]** 135 AA align

Score = 108 bits (270), Expect = 2e-23
Identities = 56/134 (41%), Positives = 79/134 (58%), Gaps = 5/134 (3%)

```
Query: 4   KGLKGVMVCLLILGLVL--EQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKL 60
           + +K ++VC+L+LGLVL  E +QVE KSCC +T  RN YN CR  GA +  C  +  CK+
Sbjct: 2   EAVKSLIVCVLVLGLVLQHEHIQVEAKSCCPSTTARNIYNSCRFTGASRDKCCKISGCKI 61

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
             G  C   F    L  +S+E D +++C LGC SSVC  M N  A +EE    V+ C +A
Sbjct: 62  VDG-KCKPPFIHHTLHPDSEESDVLDFCKLGCTSSVCSNM-NTFAGNEEGNHAVDRCNEA 119

Query: 121 CVNFCNGDAGLTSL 134
           C  FC  +A + ++
Sbjct: 120 CYRFCTNEAEIVTV 133
```

tr Q5Z5V1 Q5Z5V1_ORYSA **Putative thionin Osthi1 [OSJNBa0020P04.23] [Oryza sativa (japonica cultivar-group)]** 135 AA align

Score = 106 bits (264), Expect = 9e-23
Identities = 54/134 (40%), Positives = 82/134 (60%), Gaps = 5/134 (3%)

```
Query: 4   KGLKGVMVCLLILGLVLEQ--VQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60
           KG+K +++C+L+LGLVL+Q  ++V  KSCC TT  RN YN CR + G ++ CS +  CK+
Sbjct: 2   KGVKSLIMCVLVLGLVLQQETIKVGAKSCCPTTTARNIYNACRFAHGTRERCSKLSGCKI 61

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
             G  C   +   L   ++E D +++C LGC SSVC   +N    +EE    VE C +A
Sbjct: 62  VDG-KCKPPYIHHTLHDFAEELDVLDFCMLGCTSSVCS-NINTCKLNEEGNGAVERCNEA 119

Query: 121 CVNFCNGDAGLTSL 134
           C +FCN +A + ++
Sbjct: 120 CYHFCNKEADIVTI 133
```

tr Q5Z434 Q5Z434_ORYSA **Putative thionin Osthi1 [OSJNBa0061G23.50] [Oryza sativa (japonica cultivar-group)]** 135 AA align

Score = 105 bits (261), Expect = 2e-22
Identities = 51/129 (39%), Positives = 80/129 (61%), Gaps = 5/129 (3%)

```
Query: 4   KGLKGVMVCLLILGLVLEQ--VQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60
           +G+K +++C+L+LGLVL+Q  +QVE KSCC +T  RN YN CR + G++  C+  +  CK+
Sbjct: 2   EGVKSLIMCMLVLGLVLQQEKIQVEAKSCCPSTTARNVYNSCRFAGGSRDTCAKLSGCKI 61

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
             G +C   +   L   ++E + +++C LGC SSVC  M +  + +EE    V+ C DA
Sbjct: 62  VDG-NCKPPYVHHTLHPEAEESEVLDFCKLGCASSVCSTM-STLSSNEEANYAVDRCNDA 119
```

```
Query: 121 CVNFCNGDA 129
           C  FC  +A
Sbjct: 120 CHRFCTKEA 128
```

tr Q5Z4S4 Q5Z4S4_ORYSA **Putative thionin Osthi1 [P0597A07.39] [Oryza sativa (japonica cultivar-group)]** 135 AA align

```
 Score =  102 bits (255), Expect = 1e-21
 Identities = 51/129 (39%), Positives = 79/129 (60%), Gaps = 5/129 (3%)

Query: 4   KGLKGVMVCLLILGLVLEQ--VQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60
           +G+K +++C+L+LGLVL+Q  +QVE KSCC +T  RN YN CR + G++  C+ +  CK+
Sbjct: 2   EGVKSLIMCMLVLGLVLQQEKIQVEAKSCCPSTTARNVYNSCRFAGGSRNTCAKLSGCKI 61

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
             G +C   +    L   ++E + +++C LGC SSVC  M    A +EE    V+ C +A
Sbjct: 62  VDG-NCEPPYVHHTLHPEAEESEVVDFCKLGCASSVCSTMSTLFA-NEEANHAVDRCNEA 119

Query: 121 CVNFCNGDA 129
           C  FC  +A
Sbjct: 120 CRRFCTKEA 128
```

tr Q8LT04 Q8LT04_ORYSA **Thionin Osthi1 [Osthi1] [Oryza sativa (japonica cultivar-group)]** 135 AA align

```
 Score =  102 bits (254), Expect = 1e-21
 Identities = 51/129 (39%), Positives = 79/129 (60%), Gaps = 5/129 (3%)

Query: 4   KGLKGVMVCLLILGLVLEQ--VQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60
           +G+K +++C+L+LGLVL+Q  +QVE KSCC +T  RN YN CR + G++  C+ +  CK+
Sbjct: 2   EGVKSLIMCMLVLGLVLQQEKIQVEAKSCCPSTTARNVYNSCRFAGGSRDTCAKLSGCKI 61

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
             G +C   +    L   ++E + +++C LGC SSVC  M    A +EE    V+ C +A
Sbjct: 62  VDG-NCKPPYVHHTLHPEAEESEVVDFCKLGCASSVCSTMSTLFA-NEEANHAVDRCNEA 119

Query: 121 CVNFCNGDA 129
           C  FC  +A
Sbjct: 120 CRRFCTKEA 128
```

tr Q5Z4K0 Q5Z4K0_ORYSA **Thionin Osthi1 [OSJNBa0022O06.36] [Oryza sativa (japonica cultivar-group)]** 135 AA align

```
 Score =  101 bits (252), Expect = 2e-21
 Identities = 50/129 (38%), Positives = 79/129 (60%), Gaps = 5/129 (3%)
```

```
Query: 4   KGLKGVMVCLLILGLVLEQ--VQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60
           +G+K +++C+L+LGLVL+Q  +QVE KSCC +T  RN YN CR + G++  C+ +  CK+
Sbjct: 2   EGVKSLIMCMLVLGLVLQQEKIQVEAKSCCPSTTARNVYNSCRFAGGSRDTCAKLSGCKI 61

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
             G +C   +    L   ++E + +++C LGC SSVC  M +    +EE    V+ C +A
Sbjct: 62  VDG-NCKPPYVHHTLHPEAEESEVVDFCKLGCASSVCSTM-STLFGNEEANHAVDRCNEA 119

Query: 121 CVNFCNGDA 129
           C  FC  +A
Sbjct: 120 CRRFCTKEA 128
```

tr Q9S975 Q9S975_CRAAB **Crambin PRECURSOR=THIONIN variant THI2CA11 (Fragment) [Crambe abyssinica (Abyssinian crambe)]** 130 AA align

```
 Score = 90.1 bits (222), Expect = 7e-18
 Identities = 48/120 (40%), Positives = 68/120 (56%), Gaps = 5/120 (4%)

Query: 9   VMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCP 67
           V++ +LI+ LV+ Q QVE KSCC +   RN YN+CR  G   + +C+++  CK+ S   CP
Sbjct: 2   VILSVLIMSLVIAQNQVEAKSCCPSITARNTYNVCRLPGTPRPVCASISGCKILSVTKCP 61

Query: 68  KGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD--EEMKLYVENCGDACVNFC 125
              P   L+++ D  +  EYC LGC SSVC  +      D  E +   V  C +AC NFC
Sbjct: 62  SNLPYENLKNSGDVVN--EYCKLGCASSVCGALTTLQNSDASEVVDGAVAQCTNACSNFC 119
```

tr Q43225 Q43225_TULGE **Thionin class 1 precursor [Thi1-2] [Tulipa gesneriana (Tulip)]** 114 AA align

```
 Score = 90.1 bits (222), Expect = 7e-18
 Identities = 51/121 (42%), Positives = 64/121 (52%), Gaps = 22/121 (18%)

Query: 10  MVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCPK 68
           M+ ++ILGLV+ Q QVE KSCCR T  RNCYN+CR  G   + +C+  C CK+ S   CP
Sbjct: 1   MMVVVILGLVVAQTQVEAKSCCRNTTARNCYNVCRLPGTPRPVCAATCDCKIISSGKCPP 60

Query: 69  GFPKLALESNSDEPDTIEYCNLGCRSSVCDY--MVNAAAD--DEEMKLYVENCGDACVNF 124
           G+ K                LGC SS C    +V+ A D   E MK  VE C +AC
Sbjct: 61  GYEK----------------LGCASSTCSTIDVVDEALDVAKEVMKEAVERCNNACSEV 103

Query: 125 C 125
           C
Sbjct: 104 C 104
```

sp Q9SBK8 THN_BRARP **Thionin precursor [THI2] [Brassica rapa subsp. pekinensis (Chinese cabbage) (Celery cabbage)]** 133 AA align

```
 Score = 89.4 bits (220), Expect = 1e-17
 Identities = 48/121 (39%), Positives = 67/121 (54%), Gaps = 4/121 (3%)

Query: 7   KGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKL-CSTVCRCKLTSGLS 65
           K V++ ++I+ LV+ Q QVE K CC  T+ RN YN CR  GA    C+ +  CK+ SG +
Sbjct: 4   KTVILGVIIMSLVMAQNQVEAKICCPRTIDRNIYNACRLTGASMTNCANLSGCKIVSGTT 63

Query: 66  CPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDE-EMKLYVENCGDACVNF 124
           CP G+    L++  D  +  EYC LGC SSVC  +       +   +  V  C +AC NF
Sbjct: 64  CPPGYTHDILQNYGDAVN--EYCKLGCASSVCGALTTLKNSMQVNCEGAVSQCTNACSNF 121

Query: 125 C 125
           C
Sbjct: 122 C 122
```

tr Q43224 Q43224_TULGE **Thionin class 1 precursor [Thi1-1] [Tulipa gesneriana (Tulip)]** 121 AA align

```
 Score = 87.8 bits (216), Expect = 3e-17
 Identities = 51/118 (43%), Positives = 69/118 (58%), Gaps = 9/118 (7%)

Query: 10  MVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGA-QKLCSTVCRC-KLTSGLSCP 67
           M+ ++ILGLV+ Q QVE KSCCRTT  RNCYN+CR  G  Q LC+  C C  +T+G +CP
Sbjct: 1   MMVVVILGLVVAQTQVEAKSCCRTTAARNCYNVCRLGGTPQTLCARTCDCIHITTG-NCP 59

Query: 68  KGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVNFC 125
           +  PKL   S++     ++   L     V D +++ A   E MK  VE C +AC   C
Sbjct: 60  RSHPKLGSASSTSSTTNVDDEAL----DVVDEVLDVA--KEAMKEAVERCNNACSEVC 111
```

tr Q9S976 Q9S976_CRAAB **Crambin PRECURSOR=THIONIN variant THI2CA10 (Fragment) [Crambe abyssinica (Abyssinian crambe)]** 134 AA align

```
 Score = 86.3 bits (212), Expect = 1e-16
 Identities = 47/122 (38%), Positives = 68/122 (55%), Gaps = 5/122 (4%)

Query: 7   KGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLS 65
           K V++ +LI+ LV+ Q QVE KSCC +   RN YN+CR  G  + +C+T+  C + S  +
Sbjct: 4   KSVILSVLIISLVMAQNQVEAKSCCPSITARNTYNICRLPGTPRPVCATLSGCIIQSDST 63

Query: 66  CPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKL--YVENCGDACVN 123
           C   +P   L+++ D  +  EYC LGC SSVC  +       +   +  V  C +AC N
Sbjct: 64  CKPPYPYGDLKNSGDAVN--EYCKLGCASSVCGSLATFQNSDASKIVDGAVAQCTNACSN 121

Query: 124 FC 125
           FC
Sbjct: 122 FC 123
```

tr Q43226 **Thionin class 1 precursor [Thi1-3] [Tulipa gesneriana** 107

Q43226_TULGE **(Tulip)]** AA align

Score = 85.9 bits (211), Expect = 1e-16
Identities = 47/117 (40%), Positives = 61/117 (51%), Gaps = 21/117 (17%)

```
Query: 10  MVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCPK 68
           M+ ++ILGLV+ Q QVE KSCCR T  RNCYN+CR  G  + +C+  C CK+ S   CP
Sbjct: 1   MMVVVILGLVVAQTQVEAKSCCRNTTARNCYNVCRLPGTPRPVCAATCDCKIISSGKCPP 60

Query: 69  GFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVNFC 125
           G+ KL                    S V D  ++ A  +E MK  VE C +AC   C
Sbjct: 61  GYEKLGF------------------SDVADEALDVA--EEVMKEAVERCNNACSEVC 97
```

tr Q9S981 **Crambin PRECURSOR=THIONIN variant THI2CA2 (Fragment)** 134
Q9S981_CRAAB **[Crambe** AA
abyssinica (Abyssinian crambe)] align

Score = 85.1 bits (209), Expect = 2e-16
Identities = 48/122 (39%), Positives = 65/122 (52%), Gaps = 5/122 (4%)

```
Query: 7   KGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQ-KLCSTVCRCKLTSGLS 65
           K V++ +LI+ LV+   QVE KSCC T + R  Y +CR  G+    C     C   SG
Sbjct: 4   KIVILSVLIMNLVMAHNQVEAKSCCPTPIARKTYVVCRLTGSTIASCIKYSGCITISGTQ 63

Query: 66  CPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD--EEMKLYVENCGDACVN 123
           CP G+P   L ++ D  +  EYC LGC SSVC  +      D  E + + V +C +AC N
Sbjct: 64  CPNGYPHDILGNSGDAVN--EYCKLGCASSVCGALTTLKNSDASEIVNVAVAHCTNACSN 121

Query: 124 FC 125
           FC
Sbjct: 122 FC 123
```

tr Q9S974 **Crambin PRECURSOR=THIONIN variant THI2CA12 (Fragment)** 135
Q9S974_CRAAB **[Crambe** AA
abyssinica (Abyssinian crambe)] align

Score = 84.3 bits (207), Expect = 4e-16
Identities = 48/123 (39%), Positives = 68/123 (55%), Gaps = 6/123 (4%)

```
Query: 7   KGVMVCLLILGLVLEQVQVEG-KSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGL 64
           K V++ +LI+ LV+ Q QVE  KSCC T   RN +++CR  G    LC+ +  CK+ S
Sbjct: 4   KTVILSVLIMSLVMAQNQVEAAKSCCPTKSARNTFDVCRLTGTSMGLCAAISECKILSVT 63

Query: 65  SCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD--EEMKLYVENCGDACV 122
            CP   P   L+++ D  +  EYC LGC SSVC  +    + D  E +   V  C +AC
Sbjct: 64  KCPSNLPYDNLKNSGDAVN--EYCKLGCASSVCGSLTTLQSSDASEIVDGAVAQCTNACS 121

Query: 123 NFC 125
           +FC
Sbjct: 122 DFC 124
```

tr Q9S9A0 Q9S9A0_VISAL **Thionin [Viscum album (European mistletoe)]** 114 AA align

Score = 83.2 bits (204), Expect = 8e-16
Identities = 44/118 (37%), Positives = 64/118 (53%), Gaps = 23/118 (19%)

```
Query: 9   VMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCP 67
           +++ +L+LG +L    VE KSCCR T GRNCYN CR  G  + +C+++C CK+ SG  CP
Sbjct: 9   MLLLVLLLGALLVS-SVESKSCCRNTTGRNCYNACRVPGTPRPVCASLCDCKIISGSKCP 67

Query: 68  KGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVNFC 125
             +P+              YC LGC+S+ C    N+  D E ++     C  AC + C
Sbjct: 68  ADYPRF-------------YCTLGCQSTQC---ANSNGDAEAVR-----CKTACSDLC 104
```

sp Q42597 THN22_ARATH **Thionin 2.2 precursor [THI2.2] [Arabidopsis thaliana (Mouse-ear cress)]** 134 AA align

Score = 82.8 bits (203), Expect = 1e-15
Identities = 51/124 (41%), Positives = 64/124 (51%), Gaps = 5/124 (4%)

```
Query: 7   KGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLS 65
           K V+  LLI+ LVL Q+QVE K CC T   R+ Y +C  S  +Q  C    +CK TS
Sbjct: 4   KTVISSLLIMSLVLAQIQVEAKICCPTKDDRSVYFVCMLSVSSQFYCLLKSKCKNTSQTI 63

Query: 66  CPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKL--YVENCGDACVN 123
           CP G+    LE++ D  +  EYC LGC SSVC  +      D    L   VE C  AC +
Sbjct: 64  CPPGYTNDILENSGDAVN--EYCKLGCASSVCGALTTLQNFDTSKVLSEAVEQCTKACSS 121

Query: 124 FCNG 127
            C G
Sbjct: 122 VCTG 125
```

tr Q43227 Q43227_TULGE **Thionin class 1 precursor (Fragment) [Thi1-4] [Tulipa gesneriana (Tulip)]** 112 AA align

Score = 82.0 bits (201), Expect = 2e-15
Identities = 46/113 (40%), Positives = 60/113 (52%), Gaps = 13/113 (11%)

```
Query: 16  LGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCPKGFPKLA 74
           LGLV+ Q QV+ KSCC +T  RNCYN+CR  G  + +C+  C CK+ +G  CP  +PKL
Sbjct: 1   LGLVVAQTQVDAKSCCPSTAARNCYNVCRFPGTPRPVCAATCGCKIITGTKCPPDYPKLG 60

Query: 75  LES--NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVNFC 125
             +  NSD  D            V D  ++ A    E MK  VE C +AC    C
Sbjct: 61  WSTFQNSDVADK--------ALDVVDEALHVA--KEVMKEAVERCNNACSEVC 103
```

tr Q9S980 Q9S980_CRAAB **Crambin PRECURSOR=THIONIN variant THI2CA3 (Fragment) [Crambe** 133 AA

abyssinica (Abyssinian crambe)] align

Score = 80.9 bits (198), Expect = 4e-15
Identities = 49/122 (40%), Positives = 67/122 (54%), Gaps = 6/122 (4%)

```
Query: 7   KGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLS 65
           K V++ +LI+ LV+ Q QVE KSCC T   R  YN CR+ G  + +C+ +  CK+
Sbjct: 4   KTVILSVLIMTLVMAQNQVEAKSCCPTMAARIQYNACRALGTPRPVCAALSGCKILDVTK 63

Query: 66  CPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD--EEMKLYVENCGDACVN 123
           CP  +    L+S SD  +  EYC LGC SSVC  + N    D  E +   V  C +AC +
Sbjct: 64  CPPDYRYDILKS-SDALN--EYCKLGCASSVCGALANLQNSDASEIVNGAVVQCNNACSS 120

Query: 124 FC 125
           FC
Sbjct: 121 FC 122
```

tr Q9S977 **Crambin PRECURSOR=THIONIN variant THI2CA9 (Fragment)** 135
Q9S977_CRAAB **[Crambe** AA
abyssinica (Abyssinian crambe)] align

Score = 80.5 bits (197), Expect = 5e-15
Identities = 47/123 (38%), Positives = 67/123 (54%), Gaps = 6/123 (4%)

```
Query: 7   KGVMVCLLILGLVLEQVQVEG-KSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGL 64
           K V++ +LI+ LV+ Q QVE  KSC  T   RN +++CR  G    LC+ +  CK+ S
Sbjct: 4   KTVILSVLIMSLVMAQNQVEAAKSCYPTKSARNTFDVCRLTGTSMGLCAAISECKILSVT 63

Query: 65  SCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD--EEMKLYVENCGDACV 122
            CP   P   L+++ D  +  EYC LGC SSVC  +    + D  E +   V  C +AC
Sbjct: 64  KCPSNLPYNNLKNSGDAVN--EYCKLGCASSVCGSLTTLQSSDASEIVDGAVAQCTNACS 121

Query: 123 NFC 125
           +FC
Sbjct: 122 DFC 124
```

sp Q42596 **Thionin 2.1 precursor [THI2.1] [Arabidopsis thaliana** 134
THN21_ARATH **(Mouse-ear** AA
cress)] align

Score = 79.3 bits (194), Expect = 1e-14
Identities = 45/119 (37%), Positives = 66/119 (54%), Gaps = 6/119 (5%)

```
Query: 9   VMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSGLSCPK 68
           +++ LLI+ LV+ QVQVE K CC +   RN Y++CR R ++  C  V  C+ +   +CP+
Sbjct: 6   LILSLLIMSLVMAQVQVEAKICCPSNQARNGYSVCRIRFSKGRCMQVSGCQNSD--TCPR 63

Query: 69  GFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD--EEMKLYVENCGDACVNFC 125
           G+    LE+++D   T E+C LGC +SVC  M      D  E +    E C   C  FC
Sbjct: 64  GWVNAILENSADA--TNEHCKLGCETSVCGAMNTLQNSDASEIVNGASEQCAKGCSIFC 120
```

sp P01538 THN3_VISAL **Viscotoxin A3 precursor [THI2.1] [Viscum album (European mistletoe)]** 111 AA

align

Score = 77.4 bits (189), Expect = 4e-14
Identities = 45/118 (38%), Positives = 61/118 (51%), Gaps = 22/118 (18%)

```
Query: 9   VMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCP 67
           +++ +L+LG +L   QVE KSCC  T GRN YN CR  GA +  C+ +  CK+ SG +CP
Sbjct: 9   LVLLVLLLGALLVS-QVESKSCCPNTTGRNIYNACRLTGAPRPTCAKLSGCKIISGSTCP 67

Query: 68  KGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVNFC 125
             +PK              YC LGC SS C    N+  D E ++     C  AC + C
Sbjct: 68  SDYPKF-------------YCTLGCESSQC--ATNSNGDAEAVR-----CKTACSDLC 105
```

tr Q9S9A2 Q9S9A2_VISAL **Viscotoxin A [Viscum album (European mistletoe)]** 111 AA

align

Score = 77.4 bits (189), Expect = 4e-14
Identities = 47/126 (37%), Positives = 60/126 (47%), Gaps = 24/126 (19%)

```
Query: 4   KGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQ-KLCSTVCRCKLTS 62
           +G   + + LL+  LV+  V   E KSCC  T GRN YN CR  GA    C+ +  CK+ S
Sbjct: 5   RGSSFLFLVLLLGALVVSNV--ESKSCCPNTTGRNIYNACRLTGAPCPTCAKLSGCKIIS 62

Query: 63  GLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACV 122
           G +CP  +PK              YC LGC SS C    N   D E ++     C  AC
Sbjct: 63  GSTCPSDYPKF-------------YCTLGCESSQC---ANTNGDAEAVR-----CKTACS 101

Query: 123 NFCNGD 128
           + CN D
Sbjct: 102 DLCNND 107
```

tr Q9S9A1 Q9S9A1_VISAL **Thionin [Viscum album (European mistletoe)]** 115 AA

align

Score = 73.6 bits (179), Expect = 6e-13
Identities = 43/130 (33%), Positives = 63/130 (48%), Gaps = 23/130 (17%)

```
Query: 4   KGLKGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSG 63
           +G +   + LL+L   L    VE K CCR   G+ CYNLC +  + + C+  C CK  SG
Sbjct: 2   EGARASSLLLLLLLGALVVYDVESKICCRAPAGKKCYNLCTALLSSETCANTCYCKDVSG 61

Query: 64  LSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVN 123
            +CP  +P               YCNLGC+SS+C     A ++ E    +    C  AC +
Sbjct: 62  ETCPADYPAF-------------YCNLGCQSSLC-----AKSNGEAEAV---RCMTACSD 100

Query: 124 FCNGDAGLTS 133
            C   +G+T+
Sbjct: 101 LCGSGITA 108
```

tr Q9S979 Q9S979_CRAAB **Crambin PRECURSOR=THIONIN variant THI2CA5 (Fragment) [Crambe abyssinica (Abyssinian crambe)]** 118 AA align

Score = 73.2 bits (178), Expect = 8e-13
Identities = 41/112 (36%), Positives = 58/112 (51%), Gaps = 4/112 (3%)

```
Query: 22  QVQVEGKSCCRTTLGRNCYNLCRSRG-AQKLCSTVCRCKLTSGLSCPKGFPKLALESNSD 80
           Q QVE   CC  T  R+ +N+CR  G A+ +C+T   C +  G +CP  +    L++++
Sbjct: 1   QNQVEANICCPNTTARSNFNVCRLPGTAEPICATDTGCIIIPGATCPGDYANNILKNSAQ 60

Query: 81  EPDTIEYCNLGCRSSVCDYMVNAAADD--EEMKLYVENCGDACVNFC-NGDA 129
               EYC  GC SSVC  + N    D  E +   V  C +AC +FC NG A
Sbjct: 61  GNAVNEYCKWGCASSVCGALTNLQNSDAREIVNGAVRQCTNACFDFCTNGSA 112
```

sp P08943 THNB_VISAL **Viscotoxin B precursor (Fragment) [THI2.2] [Viscum album (European mistletoe)]** 103 AA align

Score = 72.4 bits (176), Expect = 1e-12
Identities = 42/113 (37%), Positives = 58/113 (51%), Gaps = 24/113 (21%)

```
Query: 25  VEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLSCPKGFPKLALESNSDEPD 83
           VE KSCC  T GRN YN CR   G+++ C+++  CK+ S  +CP  +PK
Sbjct: 4   VESKSCCPNTTGRNIYNTCRLGGGSRERCASLSGCKIISASTCPSDYPKF---------- 53

Query: 84  TIEYCNLGCRSSVCDYMV---NAAADDEEMKLYVENCGDACVNFCNGDAGLTS 133
              YC LGC+SS C  +    N+  D E ++     C  AC N C  D G+T+
Sbjct: 54  ---YCTLGCQSSKCASITTPPNSEVDAEAVR-----CKAACSNLC--DFGVTT 96
```

sp Q8VZK8 THN23_ARATH **Probable thionin 2.3 precursor [At2g15010] [Arabidopsis thaliana (Mouse-ear cress)]** 135 AA align

Score = 71.6 bits (174), Expect = 2e-12
Identities = 45/123 (36%), Positives = 65/123 (52%), Gaps = 6/123 (4%)

```
Query: 7   KGVMVCLLILGLVLEQVQVEG-KSCCRTTLGRNCYNLCRSRG-AQKLCSTVCRCKLTSGL 64
           K V+  +L++GLV+ Q+QVE  K+CC +   R  +  C S G  Q LCS    C+ T
Sbjct: 4   KTVIFSVLVMGLVISQIQVEAQKTCCPSQSTRKEFEDCISEGNLQILCSAESGCRDTYVG 63

Query: 65  SCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDE--EMKLYVENCGDACV 122
            CP GFP  +L ++ D  +   YC LGC SS+C  + +    D   ++ + VE C  AC
Sbjct: 64  YCPSGFPYGSLTNSGDVVNV--YCKLGCVSSLCGALTSLQKLDTSGKVNVAVERCTKACS 121

Query: 123 NFC 125
             C
Sbjct: 122 TIC 124
```

tr Q41609 **Thionin class 4 precursor [Thi4-1] [Tulipa gesneriana** 124

Q41609_TULGE **(Tulip)]** AA align

Score = 71.6 bits (174), Expect = 2e-12
Identities = 44/118 (37%), Positives = 60/118 (50%), Gaps = 6/118 (5%)

```
Query: 10  MVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGA--QKLCSTVCRCKLTSGLSCP 67
           M+ ++ILGLV+ Q QVE KSC  +T  + CYN CR  G   + +C+  C CK+ S  +CP
Sbjct: 1   MMVVVILGLVVAQTQVEAKSCFPSTAAKYCYNACRLPGCRPETICAARCGCKIISSGNCP 60

Query: 68  KGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVNFC 125
            G+    L S S    T    +      V D  ++ A   E MK  VE C +AC   C
Sbjct: 61  PGYDYENLGSASSTSSTSNVDDEAL--DVVDEALDVA--KEAMKEAVERCNNACSEVC 114
```

tr Q5Z554 Q5Z554_ORYSA **Putative thionin Osthi1 [OSJNBa0085C03.20] [Oryza sativa (japonica cultivar-group)]** 112 AA align

Score = 71.2 bits (173), Expect = 3e-12
Identities = 46/129 (35%), Positives = 66/129 (50%), Gaps = 28/129 (21%)

```
Query: 4   KGLKGVMVCLLILGLVLEQ--VQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60
           +G+K +++C L+LGLVL+Q  + VE KSCC +T  RN YN CR + G+++ C+ +  CK
Sbjct: 2   EGVKSLIMCALVLGLVLQQEKIHVEAKSCCPSTSVRNVYNSCRFAGGSREACAKLSTCKH 61

Query: 61  TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDA 120
             G SC   +  L L S     DT+                    A +E +   VE C DA
Sbjct: 62  FDG-SCQPPYDHLTLHS-----DTV-------------------AANEGVNAAVERCEDA 96

Query: 121 CVNFCNGDA 129
           C  FC  +A
Sbjct: 97  CDRFCTNEA 105
```

sp Q9C8D6 THN24_ARATH **Probable thionin 2.4 precursor [At1g66100] [Arabidopsis thaliana (Mouse-ear cress)]** 134 AA align

Score = 65.5 bits (158), Expect = 2e-10
Identities = 42/122 (34%), Positives = 57/122 (46%), Gaps = 5/122 (4%)

```
Query: 7   KGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLS 65
           K ++V +LI+ L + Q QV+   CC +   R  YN C  + G+   C     C  S  +
Sbjct: 4   KTLIVSVLIMSLFMAQNQVDANICCPSIQARTFYNACLFAVGSPSSCIRNSSCLDISEST 63

Query: 66  CPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD--EEMKLYVENCGDACVN 123
           CP+G+    LE+  D     EYC LGC SSVC  +      D  E E +   VE C  AC
Sbjct: 64  CPRGYTNDILENTGDA--VTEYCKLGCVSSVCGALTILQNSDASEIVNGEVEKCTMACST 121

Query: 124 FC 125
            C
Sbjct: 122 VC 123
```

tr Q5Z551 Q5Z551_ORYSA **Putative thionin Osthi1 [OSJNBa0085C03.23] [Oryza sativa (japonica cultivar-group)]** 148 AA

align

Score = 61.2 bits (147), Expect = 3e-09
Identities = 32/75 (42%), Positives = 48/75 (63%), Gaps = 4/75 (5%)

```
Query: 4  KGLKGVMVCLLILGLVLEQ--VQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60
          +G+K +++C L+LGLVL+Q  + VE KSCC +T  RN YN CR + G+++ C+ +  CK
Sbjct: 2  EGVKSLIMCALVLGLVLQQEKIHVEAKSCCPSTSARNVYNSCRFAGGSREACAKLSTCKH 61

Query: 61 TSGLSCPKGFPKLAL 75
            G SC   +  L L
Sbjct: 62 FDG-SCQPPYDHLTL 75
```

sp P07504 THN_PYRPU **Thionin [THI1] [Pyrularia pubera (Rabbitwood) (Buffalo nut)]** 47 AA

align

Score = 60.8 bits (146), Expect = 4e-09
Identities = 24/47 (51%), Positives = 33/47 (70%), Gaps = 2/47 (4%)

```
Query: 28 KSCCRTTLGRNCYNLCRSRG--AQKLCSTVCRCKLTSGLSCPKGFPK 72
          KSCCR T  RNCYN+CR  G  ++++C+  C CK+ SG +CP  +PK
Sbjct: 1  KSCCRNTWARNCYNVCRLPGTISREICAKKCDCKIISGTTCPSDYPK 47
```

sp P60057 THND_HELPU **Hellethionin D [Helleborus purpurascens (Purple hellebore)]** 46 AA

align

Score = 52.4 bits (124), Expect = 2e-06
Identities = 22/45 (48%), Positives = 27/45 (59%), Gaps = 1/45 (2%)

```
Query: 28 KSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLSCPKGFP 71
          KSCCR TL RNCYN CR + G+Q  C  +C C   +  +CP   P
Sbjct: 1  KSCCRNTLARNCYNACRFTGGSQPTCGILCDCIHVTTTTCPSSHP 45
```

sp P83554 THNC_VISAL **Viscotoxin C1 [Viscum album (European mistletoe)]** 46 AA

align

Score = 50.8 bits (120), Expect = 4e-06
Identities = 21/46 (45%), Positives = 30/46 (64%), Gaps = 1/46 (2%)

```
Query: 28 KSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLSCPKGFPK 72
          KSCC  T GRN YN CR + G+++ C+ +  CK+ S  +CP  +PK
Sbjct: 1  KSCCPNTTGRNIYNTCRFAGGSRERCAKLSGCKIISASTCPSDYPK 46
```

sp P01541 THN_DENCL **Denclatoxin B [Dendrophthora clavata (Columbian mistletoe)]** 46 AA

align

Score = 50.4 bits (119), Expect = 6e-06
Identities = 21/44 (47%), Positives = 28/44 (62%), Gaps = 1/44 (2%)

```
Query: 28 KSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCPKGF 70
          KSCC TT  RN YN+CR  G  + +C+ +  CK+ SG  CP G+
Sbjct: 1  KSCCPTTAARNQYNICRLPGTPRPVCAALSGCKIISGTGCPPGY 44
```

sp P59358 THNB_PHOLI **Ligatoxin B [Phoradendron liga (Argentine mistletoe)]** 46 AA

align

Score = 47.8 bits (112), Expect = 4e-05
Identities = 20/44 (45%), Positives = 29/44 (65%), Gaps = 1/44 (2%)

```
Query: 28 KSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCPKGF 70
          KSCC +T  RN YN CR  GA + +C+++  CK+ SG +C  G+
Sbjct: 1  KSCCPSTTARNIYNTCRLTGASRSVCASLSGCKIISGSTCDSGW 44
```

sp P01539 THN_PHOTO **Phoratoxin [Phoradendron tomentosum (California mistletoe)]** 46 AA

align

Score = 47.0 bits (110), Expect = 6e-05
Identities = 20/44 (45%), Positives = 27/44 (60%), Gaps = 1/44 (2%)

```
Query: 28 KSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLSCPKGF 70
          KSCC TT  RN YN CR   G++ +C+ +  CK+ SG  C  G+
Sbjct: 1  KSCCPTTTARNIYNTCRFGGGSRPVCAKLSGCKIISGTKCDSGW 44
```

sp P32880 THN2_VISAL **Viscotoxin A2 [THI2.3] [Viscum album (European mistletoe)]** 46 AA

align

Score = 47.0 bits (110), Expect = 6e-05
Identities = 19/41 (46%), Positives = 28/41 (67%), Gaps = 1/41 (2%)

```
Query: 28 KSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLSCP 67
          KSCC  T GRN YN CR   G++++C+++  CK+ S  +CP
Sbjct: 1  KSCCPNTTGRNIYNTCRFGGGSREVCASLSGCKIISASTCP 41
```

tr Q5Z553 Q5Z553_ORYSA **Hypothetical protein OSJNBa0085C03.21 [OSJNBa0085C03.21] [Oryza sativa (japonica cultivar-group)]** 86 AA

align

```
 Score = 46.6 bits (109), Expect = 8e-05
 Identities = 23/67 (34%), Positives = 33/67 (48%), Gaps = 1/67 (1%)

Query: 63  GLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACV 122
           G  C  G          +++ D +++C LGC SSVC   +N  A +E +   VE C  AC
Sbjct: 14  GSGCQFGLNHRRPWVKAEKSDVLDFCKLGCTSSVCS-KINTFAANEGVNAAVERCEHACD 72

Query: 123 NFCNGDA 129
            FC  +A
Sbjct: 73  RFCTNEA 79
```

sp P01540 THNA_PHOLI **Ligatoxin A [Phoradendron liga (Argentine mistletoe)]** 46 AA

align

```
 Score = 45.1 bits (105), Expect = 2e-04
 Identities = 19/44 (43%), Positives = 27/44 (61%), Gaps = 1/44 (2%)

Query: 28 KSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCPKGF 70
          KSCC +T  RN YN CR  G  +  C+++  CK+ SG +C  G+
Sbjct: 1  KSCCPSTTARNIYNTCRLTGTSRPTCASLSGCKIISGSTCBSGW 44
```

tr Q5Z4S0 Q5Z4S0_ORYSA **Thionin Osthi1-like [P0597A07.48] [Oryza sativa (japonica cultivar-group)]** 84 AA

align

```
 Score = 45.1 bits (105), Expect = 2e-04
 Identities = 20/50 (40%), Positives = 29/50 (58%), Gaps = 1/50 (2%)

Query: 80  DEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVNFCNGDA 129
           +E + +++C LGC SSVC  M    A +EE    V+ C +AC  FC  +A
Sbjct: 29  EESEVVDFCKLGCASSVCSTMSTLFA-NEEANHAVDRCNEACRRFCTKEA 77
```

sp P01537 THN1_VISAL **Viscotoxin 1-PS [THI2.4] [Viscum album (European mistletoe)]** 46 AA

align

```
 Score = 44.3 bits (103), Expect = 4e-04
 Identities = 17/41 (41%), Positives = 27/41 (65%), Gaps = 1/41 (2%)

Query: 28 KSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLSCP 67
          KSCC  T GR+ Y+ CR   G++++C+ +  CK+ S  +CP
Sbjct: 1  KSCCPBTTGRBIYBTCRFGGGSRZVCARISGCKIISASTCP 41
```

tr Q5Z557 Q5Z557_ORYSA **Hypothetical protein OSJNBa0085C03.15 [OSJNBa0085C03.15] [Oryza sativa (japonica cultivar-group)]** 78 AA

align

```
 Score = 44.3 bits (103), Expect = 4e-04
 Identities = 20/59 (33%), Positives = 37/59 (61%), Gaps = 1/59 (1%)

Query: 76  ESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVNFCNGDAGLTSL 134
           ++ ++E D +++C LGC SSVC   +N  A +EE    V++C +A   FC+ +A + ++
Sbjct: 19  DAPTEESDVLDFCKLGCTSSVCS-TINIFAVNEEGNGAVDSCNNARYRFCSKEAEVVTV 76
```

tr Q9S978 Q9S978_CRAAB **Crambin PRECURSOR=THIONIN variant THI2CA7 (Fragment) [Crambe abyssinica (Abyssinian crambe)]** 51 AA align

```
 Score = 42.0 bits (97), Expect = 0.002
 Identities = 19/46 (41%), Positives = 27/46 (58%), Gaps = 2/46 (4%)

Query: 52 CSTVCRCKLTSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVC 97
          C+ +  CK+ S   CP   P  +L+++ D  +  EYC LGC SSVC
Sbjct: 6  CAKLSSCKILSVTKCPANLPYESLKNSGDAVN--EYCMLGCASSVC 49
```

sp O88745 SCRG1_MOUSE **Scrapie-responsive protein 1 precursor (ScRG-1) [Scrg1] [Mus musculus (Mouse)]** 98 AA align

```
 Score = 35.4 bits (80), Expect = 0.19
 Identities = 26/80 (32%), Positives = 35/80 (43%), Gaps = 17/80 (21%)

Query: 6  LKGVMVCLLILGLVLEQVQVEGK--SCCRTTL-GRNCYNLCRSRGAQKL----------- 51
          +K V++ +L L L+LE   +     SC R  L  RNC+NL   R   KL
Sbjct: 2  MKSVVLVILGLTLLLETQAMPSSRLSCYRKLLKDRNCHNLPEGRADLKLIDANVQHHFWD 61

Query: 52 ---CSTVCRCKLTSGLSCPK 68
             C  +C C  +  L CPK
Sbjct: 62 GKGCEMICYCNFSELLCCPK 81
```

sp P01542 CRAM_CRAAB **Crambin [THI2] [Crambe abyssinica (Abyssinian crambe)]** 46 AA align

```
 Score = 35.0 bits (79), Expect = 0.25
 Identities = 14/40 (35%), Positives = 24/40 (60%), Gaps = 1/40 (2%)

Query: 29 SCCRTTLGRNCYNLCRSRGA-QKLCSTVCRCKLTSGLSCP 67
          +CC + + R+ +N+CR  G  + LC+T   C +  G +CP
Sbjct: 2  TCCPSIVARSNFNVCRLPGTPEALCATYTGCIIIPGATCP 41
```

sp Q9Z0K6 SCRG1_RAT **Scrapie-responsive protein 1 precursor (ScRG-1) [Scrg1] [Rattus norvegicus (Rat)]** 98 AA align

```
 Score = 34.3 bits (77), Expect = 0.43
 Identities = 26/77 (33%), Positives = 32/77 (40%), Gaps = 19/77 (24%)

Query: 11 VCLLILGLVL----EQVQVEGKSCCRTTL-GRNCYNLCRSRGAQKL-------------- 51
          V L+ILGL L    + +     SC R  L  RNC+NL   R   KL
Sbjct: 5  VVLVILGLTLLLGTQAMPSSRLSCYRKLLKDRNCHNLPEGRADLKLIDENVQHHFWEGKG 64

Query: 52 CSTVCRCKLTSGLSCPK 68
          C  +C C  +  L CPK
Sbjct: 65 CEMICYCNFSELLCCPK 81
```

tr Q9LN81 Q9LN81_ARATH **T12C24.19 [Arabidopsis thaliana (Mouse-ear cress)]** 212 AA align

```
 Score = 33.9 bits (76), Expect = 0.57
 Identities = 34/127 (26%), Positives = 50/127 (38%), Gaps = 24/127 (18%)

Query: 13  LLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSGLSCP----- 67
           L+++ LV+  + +E ++      L   CY  C       L +           L CP
Sbjct: 9   LVVMMLVMGNMLIEAEAVMSFKL---CYGGC-------LVACALIAPPIKKLFCPFLCIK 58

Query: 68  --KGFPKLALESNSDEPD-TIEYCNLGCRSSVC------DYMVNAAADDEEMKLYVENCG 118
             K  P L+ E+N +E D T  YC LGC +  C      D   N  A      K+  + C
Sbjct: 59  DCKRRPMLSFEANLNEIDQTGSYCELGCATDRCVSSSSIDDKGNLLAQTAAQKIPFKECY 118

Query: 119 DACVNFC 125
           AC+  C
Sbjct: 119 PACLVEC 125
```

tr Q7Q1J5 Q7Q1J5_ANOGA **ENSANGP00000014375 (Fragment) [ENSANGG00000011886] [Anopheles gambiae str. PEST]** 707 AA align

```
 Score = 33.5 bits (75), Expect = 0.74
 Identities = 26/99 (26%), Positives = 35/99 (35%), Gaps = 14/99 (14%)

Query: 38  NCYNLCRSRGAQKLCSTVC---RCKLTSGLSCPKGFPKLALESNSDEPDTIEYCNLGC-- 92
           N  N+C  +     CS  C   RC       C KG+   +  +NS       YC   C
Sbjct: 340 NSKNVCEPK-----CSKGCSNGRCVAPDHCECHKGYIATSSSANSKSSICTPYCKNKCVN 394

Query: 93  ----RSSVCDYMVNAAADDEEMKLYVENCGDACVNFCNG 127
               R +VC  +      D     +  C DA V+  NG
Sbjct: 395 AYCIRPNVCQCLAGHRFADNSTNVCEPICEDALVDCSNG 433
```

tr Q8W2K6 Q8W2K6_9SOLN **Proteinase inhibitor IIa [PIN2a] [Solanum americanum]** 148 AA align

```
 Score = 33.1 bits (74), Expect = 0.96
 Identities = 37/129 (28%), Positives = 52/129 (39%), Gaps = 12/129 (9%)
```

```
Query: 10  MVCLLILG-LVLEQVQVEGKSCCRTTLGRNCYNLC-RSRGA-QKLCSTVCRCKLTSGLSC 66
           + CLL+LG + L    V+ K+C R   G   Y +C RS G+ QK   T C C    G +
Sbjct: 9   LACLLVLGWMFLLAKHVDAKACTR-ECGHFSYGICPRSEGSPQKPICTNC-CSGYKGCNY 66

Query: 67  PKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVNFCN 126
                 L  E  SD P   + C   C + +      +     E K+ ++  G  C   C
Sbjct: 67  YSAKGDLICEGESD-PRNPKDCTFECDTQI----AYSKCPRSEGKMIIKPTG--CTTCCT 119

Query: 127 GDAGLTSLD 135
           G  G    D
Sbjct: 120 GYQGCYYFD 128
```

tr Q8MY77 Q8MY77_BRABE **Bb-cadherin [BbCad] [Branchiostoma belcheri (Amphoxius)]** 796 AA

align

```
 Score = 32.3 bits (72), Expect = 1.6
 Identities = 29/114 (25%), Positives = 46/114 (39%), Gaps = 13/114 (11%)

Query: 24  QVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTS-GLSCPKGFPKLALESNSDEP 82
           Q +G +C       RNCYN     G   +  T     ++    +CP+GF  ++ E + DE
Sbjct: 486 QYKGVNC---DTERNCYNFPCQNGGTCIEGTSPTPSISGRNCTCPQGFSGVSCEDDVDEC 542

Query: 83  DTIEYCNLG-CRSSVCDY--MVNAAADDEEMKLYVENCGD------ACVNFCNG 127
                C +G CR+ V  Y        D    +  ++NC +       C +F  G
Sbjct: 543 SLPNNCVVGTCRNLVGSYECTCKLGYDGYLCRNVIDNCANNPCDPGQCYSFIGG 596
```

tr Q8BKK7 Q8BKK7_MOUSE **Mus musculus 12 days embryo spinal ganglion cDNA, RIKEN full-length enriched library, clone:D130061K05 product:MEGF11 PROTEIN (KIAA1781) homolog [2410080H04Rik] [Mus musculus (Mouse)]** 947 AA

align

```
 Score = 32.0 bits (71), Expect = 2.1
 Identities = 24/84 (28%), Positives = 35/84 (41%), Gaps = 12/84 (14%)

Query: 30  CCRTTLGRNCYNLCRSRGAQKLCSTVCRCK-------LTSGLSCPKGFPKLALESNSDEP 82
           C    +G++C   C S    K C  +C+C++       +T   +C  GF     E     P
Sbjct: 652 CFPGWIGKDCSQGCPSAFFGKDCGHICQCQNGASCDHITGKCTCRTGFSGRHCEQRC-AP 710

Query: 83  DTIEYCNLGCRSSVCDYMVNAAAD 106
            T  Y   GC+  +C+ M NA  D
Sbjct: 711 GTFGY---GCQ-QLCECMNNATCD 730
```

tr Q95V69 Q95V69_TETTH **Cell surface immobilization antigen SerH6 [SerH] [Tetrahymena thermophila]** 421 AA

align

```
 Score = 31.6 bits (70), Expect = 2.8
```

```
 Identities = 12/37 (32%), Positives = 20/37 (53%)

Query: 7  KGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLC 43
          K +++CL+I  L++  +   G   C  T G+NC   C
Sbjct: 4  KTIIICLIISQLLVSVISAGGAVTCTGTTGQNCSQAC 40
```

tr Q60XC0 Q60XC0_CAEBR **Hypothetical protein CBG18730 (Fragment) [CBG18730] [Caenorhabditis briggsae]** 1168 AA align

```
 Score = 31.6 bits (70), Expect = 2.8
 Identities = 28/99 (28%), Positives = 39/99 (39%), Gaps = 10/99 (10%)

Query: 36  GRNCYNLCRSRGAQKLCSTVCRCKL---TSGLSCPKGFPKLALESNSDEPDTIEYCNLGC 92
          G  C  +C +      C+  C CKL   T+G SC    P+   +    +     CNL C
Sbjct: 545 GEKCEQICPNGLWGVDCAHKCSCKLCDPTTG-SCRCEDPERCSDGPCPDGYYGSQCNLKC 603

Query: 93  RSSV----CDYMVNAAADDEEMKLYVENCGDACVNFCNG 127
           R       CD +       +   LY +NC  +C NF  G
Sbjct: 604 RMDCLNGRCDPIFGYCTCPDG--LYGQNCEKSCPNFTFG 640
```

sp P04881 NCAP_VSVJO **Nucleocapsid protein (Nucleoprotein) [N] [Vesicular stomatitis virus (serotype New Jersey / strain Ogden)]** 422 AA align

```
 Score = 31.2 bits (69), Expect = 3.7
 Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78  NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEE 109
           N+ +PD IEY +L C S +  + V ++AD E+
Sbjct: 315 NARQPDDIEYTSLTCASLLLSFAVGSSADIEQ 346
```

tr Q89779 Q89779_9RHAB **(strain 09/82-HD-B) nucleoprotein ((strain 06/85-NM-E) nucleoprotein) ((strain 11/83-CA-B) nucleoprotein, complete cds) ((strain 01/84-SN-P1) nucleoprotein) ((strain 07/83-GA-P) Phosphoprotein and nucleocapsid genes) [Vesicular stomatitis virus]** 422 AA align

```
 Score = 31.2 bits (69), Expect = 3.7
 Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78  NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEE 109
           N+ +PD IEY +L C S +  + V ++AD E+
Sbjct: 315 NARQPDDIEYTSLTCASLLLSFAVGSSADIEQ 346
```

tr Q89594 Q89594_9RHAB **(strain 10/85-HD-B1) nucleoprotein ((strain 12/82-HD-B) nucleoprotein) [Vesicular stomatitis virus]** 422 AA

align

Score = 31.2 bits (69), Expect = 3.7
Identities = 13/32 (40%), Positives = 21/32 (65%)

```
Query: 78  NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEE 109
           N+ +PD IEY +L C S +  + V ++AD E+
Sbjct: 315 NARQPDDIEYTSLTCASLLLSFAVGSSADIEQ 346
```

tr Q89520 Q89520_9RHAB **(strain ../49-UT-B1) nucleoprotein ((strain ../52-GA-P) nucleoprotein) [Vesicular stomatitis virus]** 422 AA

align

Score = 31.2 bits (69), Expect = 3.7
Identities = 13/32 (40%), Positives = 21/32 (65%)

```
Query: 78  NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEE 109
           N+ +PD IEY +L C S +  + V ++AD E+
Sbjct: 315 NARQPDDIEYTSLTCASLLLSFAVGSSADIEQ 346
```

tr Q89040 Q89040_9RHAB **(strain ../60-PN-B) nucleoprotein [Vesicular stomatitis virus]** 422 AA

align

Score = 31.2 bits (69), Expect = 3.7
Identities = 13/32 (40%), Positives = 21/32 (65%)

```
Query: 78  NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEE 109
           N+ +PD IEY +L C S +  + V ++AD E+
Sbjct: 315 NARQPDDIEYTSLTCASLLLSFAVGSSADIEQ 346
```

tr Q89039 Q89039_9RHAB **(strain 01/85-PN-B1) nucleoprotein [Vesicular stomatitis virus]** 422 AA

align

Score = 31.2 bits (69), Expect = 3.7
Identities = 13/32 (40%), Positives = 21/32 (65%)

```
Query: 78  NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEE 109
           N+ +PD IEY +L C S +  + V ++AD E+
Sbjct: 315 NARQPDDIEYTSLTCASLLLSFAVGSSADIEQ 346
```

tr Q89038 Q89038_9RHAB **(strain 07/83-NC-P) nucleoprotein [Vesicular stomatitis virus]** 422 AA

align

Score = 31.2 bits (69), Expect = 3.7
Identities = 13/32 (40%), Positives = 21/32 (65%)

```
Query: 78  NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEE 109
           N+ +PD IEY +L C S +  + V ++AD E+
Sbjct: 315 NARQPDDIEYTSLTCASLLLSFAVGSSADIEQ 346
```

tr Q89037 Q89037_9RHAB **(strain 10/82-CR-B) nucleoprotein [Vesicular stomatitis virus]** 422 AA align

```
 Score = 31.2 bits (69), Expect = 3.7
 Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78  NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEE 109
           N+ +PD IEY +L C S +  + V ++AD E+
Sbjct: 315 NARQPDDIEYTSLTCASLLLSFAVGSSADIEQ 346
```

tr Q89036 Q89036_9RHAB **(strain 11/84-HD-B1) nucleoprotein [Vesicular stomatitis virus]** 422 AA align

```
 Score = 31.2 bits (69), Expect = 3.7
 Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78  NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEE 109
           N+ +PD IEY +L C S +  + V ++AD E+
Sbjct: 315 NARQPDDIEYTSLTCASLLLSFAVGSSADIEQ 346
```

tr Q89035 Q89035_9RHAB **(strain 10/84-GM-P) nucleoprotein [Vesicular stomatitis virus]** 422 AA align

```
 Score = 31.2 bits (69), Expect = 3.7
 Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78  NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEE 109
           N+ +PD IEY +L C S +  + V ++AD E+
Sbjct: 315 NARQPDDIEYTSLTCASLLLSFAVGSSADIEQ 346
```

tr Q89034 Q89034_9RHAB **(strain 07/84-OA-B) nucleoprotein [Vesicular stomatitis virus]** 422 AA align

```
 Score = 31.2 bits (69), Expect = 3.7
 Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78  NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEE 109
           N+ +PD IEY +L C S +  + V ++AD E+
```

```
Sbjct: 315 NARQPDDIEYTSLTCASLLLSFAVGSSADIEQ 346
```

tr Q6TXD9 Q6TXD9_9RHAB **Nucleocapsid protein [Vesicular stomatitis virus]** 422 AA

align

```
 Score = 31.2 bits (69), Expect = 3.7
 Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78  NSDEPDTIEYCNLGCRSSVCDYMVNAAADDEE 109
           N+ +PD IEY +L C S +  + V ++AD E+
Sbjct: 315 NARQPDDIEYTSLTCASLLLSFAVGSSADIEQ 346
```

tr Q9CA14 Q9CA14_ARATH **Putative phorbol ester / diacylglycerol binding protein; 61157-67783 [T32E8.13] [Arabidopsis thaliana (Mouse-ear cress)]** 1506 AA align

```
 Score = 31.2 bits (69), Expect = 3.7
 Identities = 31/121 (25%), Positives = 45/121 (36%), Gaps = 28/121 (23%)

Query: 31  CRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSG--------LSCPK--GFPKLALESNSD 80
           C+ T+ + CY L    G   LCS    C+L +G        L CPK  G  K  L S ++
Sbjct: 301 CKATVHKKCYGLLEDSGKPWLCSW---CELENGRADSERPCLLCPKKGGILKPVL-SKTE 356

Query: 81  EPDTIEYCNLGCRSSVCDYMVN-----------AAADDEEMKLYVENC---GDACVNFCN 126
               E+ +L C   + +  +              +   KL    C     AC+  CN
Sbjct: 357 NGGPAEFAHLFCSLWMPEVYIEDLKKMEPILNFPGIKETRRKLLCNLCKVKSGACIRCCN 416

Query: 127 G 127
           G
Sbjct: 417 G 417
```

tr Q620M9 Q620M9_CAEBR **Hypothetical protein CBG02727 [CBG02727] [Caenorhabditis briggsae]** 1360 AA align

```
 Score = 31.2 bits (69), Expect = 3.7
 Identities = 26/98 (26%), Positives = 35/98 (35%), Gaps = 19/98 (19%)

Query: 38   NCYNLCRSRGAQKLCSTVCRCKLTSGLSCPKGFPKLALESNSDEPDTIEY-CNLGCRSSV 96
           +C  LC+S   Q +      C+   G +           N   P T+ Y CNL C S
Sbjct: 1241 DCQGLCKSNSPQCIQGCDASCQQLCGTA----------PNPAVPLTVNYNCNLPCDSQC 1289

Query: 97   CDYMVN-------AAADDEEMKLYVENCGDACVNFCNG 127
                +       A A   E +  V +C DAC   C G
Sbjct: 1290 TQQCYHQAPTCAPACAQACEAQCPVVSCEDACQTVCKG 1327
```

tr Q84BD4 Q84BD4_MYXXA **CHP [Myxococcus xanthus]** 300 AA align

Score = 30.8 bits (68), Expect = 4.8
Identities = 22/63 (34%), Positives = 26/63 (40%), Gaps = 7/63 (11%)

```
Query: 77  SNSDEPDTIEYCN-LGCRS--SVC----DYMVNAAADDEEMKLYVENCGDACVNFCNGDA 129
           S SD P T + C  LG  S  S+C    D + N   D E   L   N  + CV  C  D
Sbjct: 58  SGSDCPSTAKTCAPLGGTSTTSICQCSTDVLCNGGTDSESTGLVCSNLDNVCVTACTSDT 117

Query: 130 GLT 132
             T
Sbjct: 118 ECT 120
```

tr Q69JY6 Q69JY6_ORYSA **Putative thaumatin-like protein [P0569E11.21] [Oryza sativa (japonica cultivar-group)]** 312 AA align

Score = 30.8 bits (68), Expect = 4.8
Identities = 29/111 (26%), Positives = 44/111 (39%), Gaps = 7/111 (6%)

```
Query: 26  EGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSGLSCPKGFPKLALESNSDEPDTI 85
           +GK  C T    +    CR  GA    +T+    L SG    K +  ++L    + P  +
Sbjct: 93  DGKGTCATGDCGSGEVECRGAGATPP-ATLVEFTLGSGGGGGKDYYDVSLVDGYNLPMVV 151

Query: 86  EYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVNF------CNGDAG 130
           E    GC ++ C   +N     E    + + C  AC  F      C+GD G
Sbjct: 152 EAAAAGCPATGCVVDLNQRCPAELKAGHGQACRSACEAFGTPEYCCSGDHG 202
```

tr Q6PTE0 Q6PTE0_MONBE **Methionine adenosyltransferase (Fragment) [Monosiga brevicollis]** 322 AA align

Score = 30.8 bits (68), Expect = 4.8
Identities = 19/74 (25%), Positives = 32/74 (42%)

```
Query: 59  KLTSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCG 118
           +L  G + PK    + + +   E  T E      R  V + +V A   D++   +++  G
Sbjct: 160 ELKDGATIPKRVHTIVISTQHSEDVTNEQLRKDLREKVINVVVPAELMDDKTVFHLQPSG 219

Query: 119 DACVNFCNGDAGLT 132
              +    GDAGLT
Sbjct: 220 RFVIGGPQGDAGLT 233
```

tr Q6PVY8 Q6PVY8_ORYSA **Zinc finger protein [Tranpr] [Oryza sativa (japonica cultivar-group)]** 302 AA align

Score = 30.4 bits (67), Expect = 6.3
Identities = 17/51 (33%), Positives = 21/51 (40%), Gaps = 3/51 (5%)

```
Query: 86  EYCNLGC---RSSVCDYMVNAAADDEEMKLYVENCGDACVNFCNGDAGLTS 133
           E+C   C     SVCD         DEE+    + CG+  V     D G TS
Sbjct: 230 EHCQFACPLCSKSVCDMSKAWERLDEELATISDTCGNKMVRILCNDCGATS 280
```

tr Q6AVT3 **Hypothetical protein OSJNBa0027J18.18 [OSJNBa0027J18.18]** 77 AA
Q6AVT3_ORYSA **[Oryza sativa (japonica cultivar-group)]** align

Score = 30.4 bits (67), Expect = 6.3
Identities = 15/54 (27%), Positives = 24/54 (43%)

```
Query: 13 LLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSGLSC 66
          LLI GLV+ +     ++ C     +  Y  C +   ++L    CRC    G+ C
Sbjct: 12 LLISGLVMLERIEHTEAVCTLFCAKGTYITCSNHPYEQLYGCACRCAPPDGVDC 65
```

tr Q95RQ1 **LD16414p (CG7447-PA, isoform A) (Cg7447-pb, isoform b)** 512
Q95RQ1_DROME **[CG7447]** AA
[Drosophila melanogaster (Fruit fly)] align

Score = 30.4 bits (67), Expect = 6.3
Identities = 22/80 (27%), Positives = 31/80 (38%), Gaps = 10/80 (12%)

```
Query: 51  LCSTVCR----CKLTSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNA--- 103
           +CS  C+    C   S  SCP GF     E + DE  T + C+  C ++   Y
Sbjct: 331 ICSARCQNGGNCTAPSTCSCPTGFTGRFCEQDVDECQTEKPCDQQCINTHGSYFCRCRQG 390

Query: 104 ---AADDEEMKLYVENCGDA 120
              +D +  K    N  DA
Sbjct: 391 FVLQSDQQSCKKVSTNADDA 410
```

tr Q7PM75 **ENSANGP00000010141 (Fragment) [ENSANGG00000007652]** 320
Q7PM75_ANOGA **[Anopheles gambiae str. PEST]** AA
align

Score = 30.4 bits (67), Expect = 6.3
Identities = 18/45 (40%), Positives = 22/45 (48%), Gaps = 9/45 (20%)

```
Query: 31  CRTTLGR------NCYNLCRSRG-AQKLCSTV--CRCKLTSGLSC 66
           C  T GR      NC  LC  RG   K+  T+  CRC+ T+G  C
Sbjct: 259 CSVTRGRRCLHPDNCATLCCGRGYTTKVIKTLEKCRCRFTNGRCC 303
```

sp P79847 **Lysozyme C precursor (EC 3.2.1.17) (1,4-beta-N-** 148
LYSC_PYGNE **acetylmuramidase C)** AA
[LYZ] [Pygathrix nemaeus (Dove langur)] align

Score = 30.0 bits (66), Expect = 8.2

```
 Identities = 30/112 (26%), Positives = 43/112 (37%), Gaps = 16/112 (14%)

Query: 10  MVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNL-CRSRGAQKLCSTVCRCKLTSGLSCPK 68
           M  L+ILGLVL  V V+GK   R  L R    L         L + VC  K  SG +
Sbjct: 1   MKALIILGLVLLSVTVQGKIFERCELARTLKKLGLDGYKGVSLANWVCLAKWESGYNTEA 60

Query: 69  GFPKLALES---------------NSDEPDTIEYCNLGCRSSVCDYMVNAAA 105
                 ES               N   P  ++ C++ C + + + + +A A
Sbjct: 61  TNYNPGDESTDYGIFQINSRYWCNNGKTPGAVDACHISCSALLQNNIADAVA 112


Database: EXPASY/UniProt
    Posted date:  Feb 16, 2005  2:07 PM
  Number of letters in database: 574,459,479
  Number of sequences in database:  1,794,555

Lambda     K      H
   0.321    0.137    0.435

Gapped
Lambda     K      H
   0.267   0.0410    0.140


Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
length of query: 136
length of database: 574,459,479
effective HSP length: 112
effective length of query: 24
effective length of database: 373,469,319
effective search space: 8963263656
effective search space used: 8963263656
T: 11
A: 40
X1: 16 ( 7.4 bits)
X2: 38 (14.6 bits)
X3: 64 (24.7 bits)
S1: 41 (21.9 bits)
S2: 66 (30.0 bits)

Wallclock time: 4 seconds
```

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```
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Please see HELP NEWS 154.
  File 654:US Pat.Full. 1976-2005/Feb 24
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  File 399:CA SEARCH(R) 1967-2005/UD=14209
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Alert feature enhanced for multiple files, etc. See HELP ALERT.
  File 349:PCT FULLTEXT 1979-2002/UB=20050217,UT=20050210
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  File 340:CLAIMS(R)/US Patent 1950-05/Feb 24
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Alerts have been run.  See HELP NEWS 347 for details.
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        (c) 2005 Amer.Chem.Soc.
*File 398: Use is subject to the terms of your user/customer agreement.
Problems with SORT.  RANK charge added. See HELP RATES 398.
  File 357:Derwent Biotech Res. _1982-2005/Feb W4
        (c) 2005 Thomson Derwent & ISI
  File  73:EMBASE 1974-2005/Feb W3
        (c) 2005 Elsevier Science B.V.
*File  73: Price change effective Jan 1, 2005. Enter HELP
RATES 73 for details.
  File  50:CAB Abstracts 1972-2005/Jan
        (c) 2005 CAB International

      Set  Items  Description
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Executing TD918
>>>SET HILIGHT: use ON, OFF, or 1-5 characters
        5311447  ALPHA
          15646  THIONIN?
      S1    373  ALPHA (2N) THIONIN?
?rd
>>>Duplicate detection is not supported for File 654.
>>>Duplicate detection is not supported for File 349.
>>>Duplicate detection is not supported for File 340.
>>>Duplicate detection is not supported for File 348.
>>>Duplicate detection is not supported for File 347.
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>>>Duplicate detection is not supported for File 342.
>>>Duplicate detection is not supported for File 398.

>>>Records from unsupported files will be retained in the RD set.
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...examined 50 records  (100)
...examined 50 records  (150)
...examined 50 records  (200)
...examined 50 records  (250)
...examined 50 records  (300)
...examined 50 records  (350)
...completed examining records
      S2     313  RD (unique items)
?e edta

Ref   Items   RT  Index-term
E1   279736    7 *EDTA
E2        1       EDTA (3-)
E3        1       EDTA + FERRIC CITRATE
E4        1       EDTA & GROWTH & REPRODUCTION
E5        1       EDTA ABRASION
E6        1       EDTA ABSORPTION ION EXCHANGE
E7        1       EDTA ACETIC ACID CALCIUM CHLORIDE HYDROGEN PER
E8        1       EDTA ACID CITRATE DEXTROSE SODIUM CITRATE HEPA
E9        1       EDTA ACTION
E10       2       EDTA ADDITION
E11       1       EDTA ADP REDUCTION AGGREGATION
E12       1       EDTA ADP-RIBOSYLTRANSFERASE

          Enter P or PAGE for more
?s e1 or e2 or e9 or e10
          279736  EDTA
               1  EDTA (3-)
               1  EDTA ACTION
               2  EDTA ADDITION
      S3  279736  'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION'
?p

Ref   Items  Index-term
E13       1  EDTA ALGINIC-ACID HUMIC-ACID NITRILOTRIACETIC-
E14       1  EDTA ALPHA AMYLASE ACTIVITY AMINO-ACIDS/
E15       1  EDTA ALPHA AMYLASE AMMONIA CITRIC BUFFER AGAR
E16       1  EDTA AMINOCHROME NORADRENOCHROME
E17       1  EDTA AMMONIUM ACETATE AMMONIUM OXALATE SODIUM
E18       1  EDTA AMMONIUM ACETATE PH ORGANIC MATTER
E19       1  EDTA AMMONIUM CARBONATE AMMONIUM ACETATE DITHI
E20       1  EDTA AMMONIUM CARBONATE AMMONIUM ACETATE NUTRI
E21       1  EDTA AMMONIUM CHLORIDE SUN LIGHT INSOLATION TE
E22       1  EDTA AMMONIUM COMPLEXES
E23       1  EDTA AMMONIUM HYDROXIDE SODIUM CHLORIDE BEHAVI
E24       1  EDTA AMMONIUM ION POLLUTION INDICATOR GROWTH I

          Enter P or PAGE for more
?p

Ref   Items  Index-term
E25       1  EDTA AMMONIUM SALT
E26       1  EDTA AMP MAGNESIUM
E27       1  EDTA AMP MANGANESE ADENYLATE KINASE
E28       1  EDTA AMPLIFIED CELL DEPLETION FOCAL ATYPIAS PR
E29       1  EDTA AMYLAMINE SALT
E30       1  EDTA ANALOG
E31       1  EDTA ANALYSIS
E32       1  EDTA AND ASCORBIC ACID ON IRON ABSORPTION
E33       1  EDTA AND DTPA
E34       1  EDTA AND MAGNESIUM
E35       1  EDTA AND SOMATOTROPIN AFFECT CALCAEMIC AND PAR
```

```
E36        1  EDTA ANILINE HYDROXYLATION AMINOPYRINE N DEMET

           Enter P or PAGE for more
?p

Ref    Items  Index-term
E37        1  EDTA ANTI COAGULATION
E38        1  EDTA ANTIBODY INTESTINAL CALCIUM BINDING PROTE
E39        1  EDTA ANTICOAGULANT
E40        1  EDTA ANTICOAGULANT AGENT BLOOD COLLECTION METH
E41        2  EDTA ANTICOAGULATION
E42        1  EDTA ANTIDOTE HEMO GLOBIN CASEIN FIBRINOGEN CO
E43        1  EDTA ANTIDOTE METABOLIC-DRUG
E44        1  EDTA ANTIDOTE METABOLIC-DRUG GLUTATHIONE HEMO
E45        1  EDTA ANTIDOTE METABOLIC-DRUG METAL TOXICITY
E46        1  EDTA ANTIDOTE METABOLIC-DRUG PHARMACO KINETICS
E47        1  EDTA ANTIDOTE-DRUG
E48        1  EDTA ANTIDOTE-DRUG CHELATING AGENT USE PEDIATR

           Enter P or PAGE for more
?p

Ref    Items  Index-term
E49        1  EDTA ANTIDOTE-DRUG EGYPTIAN POLYVALENT ANTI VE
E50        1  EDTA ANTIDOTE-DRUG INCREASED ZINC LEVELS LOWER
?e e1

Ref    Items Type  RT  Index-term
R1     53755        7 *EDTA
R2     35601   X   66  EDETIC ACID
R3         0   F    1  ETHYLENEDIAMINETETRAACETIC ACID
R4      1146   B   10  ANTICOAGULANTS
R5       989   B   11  CHELATING AGENTS
?s r1
      S4    53755  'EDTA'
?e r3

Ref    Items Type  RT  Index-term
R1        12        5 *ETHYLENEDIAMINETETRAACETIC ACID
R2         0   S    3  (ETHYLENEDINITRILO)TETRAACETIC ACID
R3     35601   X   65  EDETIC ACID
R4      7584   U    4  EDTA
?s r1-r4
              12  ETHYLENEDIAMINETETRAACETIC ACID
               0  (ETHYLENEDINITRILO)TETRAACETIC ACID
           35601  EDETIC ACID
            7584  EDTA
      S5    44607  R1-R4
?ds

Set     Items   Description
S1        373   ALPHA (2N) THIONIN?
S2        313   RD (unique items)
S3     279736   'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION'
S4      53755   'EDTA'
S5      44607   R1-R4
?s s2 and (s3 or s4 or s5 or edta? or ethylenediamine?)
             313  S2
          279736  S3
           53755  S4
           44607  S5
          280289  EDTA?
          198685  ETHYLENEDIAMINE?
      S6      79  S2 AND (S3 OR S4 OR S5 OR EDTA? OR ETHYLENEDIAMINE?)
?pause
?s s6/2002:2005
>>>One or more prefixes are unsupported
```

>>> or undefined in one or more files.
>>>Year ranges not supported in one or more files
              79 S6
        15341873 PY=2002 : PY=2005
      S7   51 S6/2002:2005
?s s6 not s7
              79 S6
              51 S7
      S8   28 S6 NOT S7
?t s8/6,kwic/all
>>>KWIC option is not available in file(s): 398, 399

8/6,KWIC/1 (Item 1 from file: 654)
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4569339 **IMAGE Available
Derwent Accession: 1995-269272
Utility
C/ **Treatment for juvenile diabetes**

Fulltext Word Count: 5801
Number of Claims: 15
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 5
Number of Figures: 9
Number of US cited patent references: 2
Number of non-US cited patent references: 1
Number of non-patent cited references: 27

Description of the Invention:
...Y. The TFG[alpha] transgenic line MT-42 used, which expresses high levels of TFG[**alpha**] from a **metallothionine** promoter, is described in Jhappan et al, Cell, 61:1137-1146 (1990...CsCl gradient purification, and dialyzed extensively against injection buffer (5 mM NaCl; 0.1 mM **EDTA** ; 5 mM Tris-HCl pH 7.4). Fertilized oocytes from FVB inbred mice (Taconic Farms...

8/6,KWIC/2 (Item 2 from file: 654)
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4525836 **IMAGE Available
Derwent Accession: 2001-407325
Utility
C/ **Trabecular meshwork induced glucocorticoid response (TIGR) fusion protein**
; POLYPEPTIDE ASSOCIATED WITH PROPER EYE FUNCTION; FOR THE DIAGNOSIS OF VISION DEFECTS

Fulltext Word Count: 13759
Number of Claims: 8
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 4
Number of Figures: 4
Number of US cited patent references: 11
Number of non-US cited patent references: 6
Number of non-patent cited references: 36

Description of the Invention:
...other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as **EDTA** ; and sugar alcohols such as mannitol or sorbitol...pattern of induction in HTM cells was distinguishable from other steroid induced proteins such as **metallothionine** , **alpha** [sub]1 -acid glycoprotein, and TAT which were maximally induced by one day dexamethasone treatment...

8/6,KWIC/3 (Item 3 from file: 654)

4461438 **IMAGE Available
Derwent Accession: 1996-230600
Utility
C/ **Neutralization of food allergens by thioredoxin**
; CONTACTING PROTEIN WITH AMOUNT OF THIOREDOXIN, NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE-THIOREDOXIN REDUCTASE AND NADPH OR AMOUNT OF THIOREDOXIN AND DITHIOTHREITOL EFFECTIVE FOR DECREASING ALLERGENICITY OF PROTEIN; ADMINISTERING

Fulltext Word Count: 29804
Number of Claims: 5
Exemplary or Independent Claim Number(s): 3
Number of Drawing Sheets: 6
Number of Figures: 6
Number of US cited patent references: 3
Number of non-US cited patent references: 3
Number of non-patent cited references: 60

Summary of the Invention:
...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

Description of the Invention:
... **Purothionin** [**alpha**] from bread wheat and **purothionins** [**alpha**]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [**alpha**] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [**alpha**]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7...same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** [**alpha**], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[**alpha**], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[**alpha**] from bread wheat, two purothionins previously not examined (**purothionins** [**alpha**]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [**alpha**]-type) by the SDS-PAGE fluorescence procedure...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two [**alpha**]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/6,KWIC/4 (Item 4 from file: 654)

4450551 **IMAGE Available
Derwent Accession: 1998-216927
Utility
C/ Manipulating nitrosative stress to kill pathologic microbes, pathologic helminths and pathologically, proliferating cells or to upregulate nitrosative stress defenses
; ALPHA-ALKYL-S-ALKYL-HOMOCYSTEINE SULFOXIMINES WHEREIN ALPHA-ALKYL CONTAINS 2 TO 8 CARBON ATOMS AND S-ALKYL CONTAINS 1 TO 10 CARBON ATOMS EXCEPT FOR ALPHA-ETHYL-METHIONINE SULFOXIMINE

Fulltext Word Count: 28487
Number of Claims: 15
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 2
Number of Figures: 2
Number of US cited patent references: 28
Number of non-US cited patent references: 1
Number of non-patent cited references: 30

Summary of the Invention:
...alkyl contains 1 to 10 carbon atoms. Some examples of species of this genus are [**alpha**]-ethyl- **buthionine** sulfoximine, [**alpha**]-propyl- **buthionine** sulfoximine, [**alpha**]-isopropyl- **buthionine** sulfoximine, and [**alpha**]-tert butyl- **buthionine** sulfoximine...

Description of the Invention:
...A preferred selective inhibitor of glutathione synthesis within the above-described genus is [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine, which can be readily prepared by the method described above, i.e., by...

...and sodium cyanide to form the corresponding hydantoin, hydrolyzing the hydantoin in alkali to form [**alpha**]-ethyl-DL- **buthionine** , converting that compound to the corresponding sulfoximine by reaction with sodium azide in sulfuric acid...

...Other sulfoximines embraced by the genus described above, include, for example, [**alpha**]-propyl-DL- **buthionine** -SR-sulfoximine, [**alpha**]-isopropyl-DL- **buthionine** -SR-sulfoximine, [**alpha**]-butyl-DL- **buthionine** - SR-sulfoximine, [**alpha**]-tert butyl-DL- **buthionine** -SR-sulfoxime, [**alpha**]-ethyl-S-butyl-[delta]-thionorvaline sulfoximine, and [alpha]-propyl-S-butyl-[delta]-thionorvaline sulfoximine. Also... where the [alpha]-alkyl is ethyl and/or the S-alkyl is butyl, e.g., [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine, with the dosages and routes of administration described above for these applying here...mM MgCl[sub]2, 7.5 mM ATP, 7.5 mM phosphoenolpyruvate, 0.3 mM **EDTA** , 10 mM L-glutamate, 10 mM L-[alpha]-aminobutyrate (an L-cysteine analog), 0.3... 2, 142 mM KCl, 36 mM MgCl[sub]2, 10 mM ATP, 0.4 mM **EDTA** , various amounts of sulfoximine, and gamma-GCS. At intervals, 50 [mu]l aliquots were removed...

...inactivation in 30 min. A similar level of inactivation was achieved with 2 mM with [**alpha**]-ethyl-DL- **buthionine** -SR-sulfoximine (DL-SR-[alpha]-ethyl-BSO), synthesized by the method described in Griffith, O...but only the L-S isomer is active as an enzyme inhibitor. The concentration of [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine is [difference]1/4 of the total concentration or [difference]500 [mu]M...

...large to bind to that enzyme (see said FIG. 5 mentioned above). In separate studies, [**alpha**]-ethyl-DL- **buthionine** -SR-sulfoximine was compared to L-buthionine-S-sulfoximine as an inhibitor of mammalian gamma ...

...protocol similar to that described here for the E. coli enzyme, it was found that [**alpha**]-ethyl-DL- **buthionine** -SR-sulfoximine was 0.025% as effective as L-buthionine-S-sulfoximine as an inhibitor of the mammalian enzyme. Expressed on the basis of the active isomer, [**alpha**]-ethyl-L-

buthionine -S-sulfoximine, [**alpha**]-ethyl-BSO has about 0.1% the inhibitory activity of L-buthionine-S-sulfoximine with...A patient with E. coli caused gastroenteritis with bloody diarrhea is administered [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine (hereinafter [alpha]-ethyl BSO) orally at a dose of 10 mmol/kg body...

...day. Bloody diarrhea resolved over four days. Similar results obtained using the same dose of [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine given intravenously at the same dose...

Non-exemplary or Dependent Claim(s):
...3. A sulfoximine as claimed in claim 2 which is [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine...

...A sulfoximine as claimed in claim 1 which is selected from the group consisting of [**alpha**]-ethyl- **buthionine** sulfoximine, [**alpha**]-propyl- **buthionine** sulfoximine, [**alpha**]-isopropyl- **buthionine** sulfoximine, [**alpha**]-butyl- **buthionine** sulfoximine, and [**alpha**]-tert butyl- **buthionine** sulfoximine...

8/6,KWIC/5 (Item 5 from file: 654)

4417299
Derwent Accession: 2001-060016
Utility
C/ **Methods for the diagnosis of glaucoma**
; NUCLEIC ACID HAVING DEFINED NUCLEOTIDE SEQUENCE WHICH CODES FOR TRABECULAR MESHWORK INDUCED GLUCOCORTICOID RESPONSE PROTEIN

Fulltext Word Count: 13915
Number of Claims: 14
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 4
Number of Figures: 4
Number of US cited patent references: 19
Number of non-US cited patent references: 9
Number of non-patent cited references: 35

Description of the Invention:
...other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as **EDTA** ; and sugar ... pattern of induction in HTM cells was distinguishable from other steroid induced proteins such as **metallothionine** , **alpha** [sub]1 -acid glycoprotein, and

8/6,KWIC/6 (Item 6 from file: 654)

4413998
Derwent Accession: 1992-415779
Utility
C/ **Antipathogenic peptides and compositions containing same**
; NUCLEOTIDE SEQUENCES CODING AN AMINO ACID SEQUENCE ASSOCIATED WITH THE PREVENTION OF INFECTION BY FUNGAL AND BACTERIAL PLANT PARASITES

Fulltext Word Count: 14201
Number of Claims: 23
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 1
Number of Figures: 1
Number of US cited patent references: 2
Number of non-patent cited references: 10

Description of the Invention:
...such as dithiothreitol to minimize oxidation of cystein residues, and a metal chelater such as **EDTA** to prevent exposure of the protein to heavy metal ions that might inactivate the protein...suitable non-ionic surfactants are the water-soluble adducts of polyethylene oxide with polypropylene glycol, **ethylenediamine** propylene glycol and alkylpolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain...volumes of a buffer solution (80 ml) comprising 0.1 M Tris-HCl, 10 mM **EDTA** , pH 7.5...For control purposes two standards are used in the above test that is [**alpha**]1/[beta] **thionin** from wheat endosperm and LT26-thionin from barley leaves...is maintained on a callus growth medium comprised of MS major, minor salts and Fe- **EDTA** (Gibco # 500-1117; 4.3 g/l), MS vitamins, 100 mg/l myo-inositol, 20...

8/6,KWIC/7 (Item 7 from file: 654)

4377970
Derwent Accession: 1993-152468
Utility
C/ **Use of thiol redox proteins for reducing protein intramolecular disulfide bonds, for improving the quality of cereal products, dough and baked goods and for inactivating snake, bee and scorpion toxins**

Fulltext Word Count: 26012
Number of Claims: 7
Exemplary or Independent Claim Number(s): 7
Number of Drawing Sheets: 53
Number of Figures: 63
Number of US cited patent references: 3
Number of non-US cited patent references: 3
Number of non-patent cited references: 44

Summary of the Invention:
...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

Description of the Drawings:
...FIG. 5 is a graph showing the effect of **purothionin** [**alpha**] and CM-1 [alpha]-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...

Description of the Invention:
...Purothionin a from bread wheat and **purothionins** [**alpha**]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [**alpha**] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [**alpha**]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7 ...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[**alpha**], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124: 237-240). However, in contrast to **purothionin** -[**alpha**] from bread wheat, two purothionins previously not examined (**purothionins** [**alpha**]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [**alpha**]-type) by the SDS-PAGE fluorescence procedure (FIG. 7...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two [**alpha**]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours.

The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...mM Tris-HC 1, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/6,KWIC/8 (Item 8 from file: 654)

4377417
Derwent Accession: 1993-152468
Utility
C/ **Use of thiol redox proteins for reducing protein intramolecular disulfide bonds, for improving the quality of cereal products, dough and baked goods and for inactivating snake, bee and scorpion toxins**

Fulltext Word Count: 28198
Number of Claims: 43
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 53
Number of Figures: 63
Number of US cited patent references: 3
Number of non-US cited patent references: 3
Number of non-patent cited references: 52

Summary of the Invention:
...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area,

Description of the Drawings:
...FIG. 5 is a graph showing the effect of **purothionin** [**alpha**] and CM-1 [alpha]-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...

Description of the Invention:
... **Purothionin** [**alpha**] from bread wheat and **purothionins** [**alpha**]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [**alpha**] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [**alpha**]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...reaction was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing mM **EDTA** and 16% glycerol in a final volume of ...5, conditions were as in FIG. 4 except that the DSG proteins were omitted and **purothionin** [**alpha**], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[**alpha**], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[**alpha**] from bread wheat, two purothionins previously not examined (**purothionins** [**alpha**]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [**alpha**]-type) by the SDS-PAGE fluorescence procedure (FIG. 7...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two [**alpha**]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and

NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/6,KWIC/9 (Item 9 from file: 654)

4331037
Derwent Accession: 1998-482938
Utility
C/ **Production process of cross-linked polyaspartic acid resin ; HYDROLYSING A CROSSLINKED POLYSUCCINIMIDE**

Fulltext Word Count: 35157
Number of Claims: 15
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 1

Description of the Invention:
...be mentioned. Their specific examples can include polyamines, for example, aliphatic polyamines such as hydrazine, **ethylenediamine** , propylenediamine, 1,4-butanediamine, pentamethylenediamine, hexamethylenediamine, heptamethylenediamine, octamethylenediamine, nonamethylenediamine, decamethylenediamine, undecamethylenediamine, dodecamethylenediamine, tetradecamethylenediamine, hexadecamethylenediamine, 1...diamino-7-hydroxyazelaic acid, isolysine, 3,5-diaminohexanoic acid, [alpha],[gamma]-diaminobutyric acid, djenkolic acid, **cystathionine** , cystine disulfoxide, [**alpha**],[epsilon]-diamino-[beta]-hydropimelic acid, hypusine, [gamma]-hydroxyornithine, [alpha]-hydroxylysine, lanthionine, lysinonorleucine, lysovitoxine, and loseanine...are those having less odor and high reactivity with imide rings in polysuccinimide, that is, **ethylenediamine** , propylenediamine, 1,4-butanediamine, heptamethylenediamine, hexamethylenediamine, and cystamine. In addition, protein-constituting amino acids such...

8/6,KWIC/10 (Item 10 from file: 654)

4314235
Derwent Accession: 1998-216927
Utility
C/ **Manipulating nitrosative stress to kill pathologic microbes, pathologic helminths and pathologically proliferating cells or to upregulate nitrosative stress defenses**

Fulltext Word Count: 30970
Number of Claims: 66
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 2
Number of Figures: 2
Number of US cited patent references: 25
Number of non-US cited patent references: 1
Number of non-patent cited references: 34

Summary of the Invention:
...alkyl contains 1 to 10 carbon atoms. Some examples of species of this genus are [**alpha**]-ethyl- **buthionine** sulfoximine, [**alpha**

]-propyl- **buthionine** sulfoximine, [**alpha**]-isopropyl- **buthionine** sulfoximine, and [**alpha**]-tert butyl- **buthionine** sulfoximine

Description of the Invention:
...A preferred selective inhibitor of glutathione synthesis within the above-described genus is [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine, which can be readily prepared by the method described above, i.e., by...

...and sodium cyanide to form the corresponding hydantoin, hydrolyzing the hydantoin in alkali to form [**alpha**]-ethyl-DL- **buthionine** , converting that compound to the corresponding sulfoximine by reaction with sodium azide in sulfuric acidOther sulfoximines embraced by the genus described above, include, for example, [**alpha**]-propyl-DL- **buthionine** -SR-sulfoximine, [**alpha**]-isopropyl-DL- **buthionine** -SR-sulfoximine, [**alpha**]-butyl-DL- **buthionine** -SR-sulfoximine, [**alpha**]-tert butyl-DL- **buthionine** -SR-sulfoxime, [**alpha**]-ethyl-S-butyl-[delta]-thionorvaline sulfoximine, and [alpha]-propyl-S-butyl-[delta]-thionorvaline sulfoximine. Also...where the [alpha]-alkyl is ethyl and/or the S-alkyl is butyl, e.g., [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine, with the dosages and routes of administration described above for these applying here...mM MgCl[sub]2, 7.5 mM ATP, 7.5 mM phosphoenolpyruvate, 0.3 mM **EDTA** , 10 mM L-glutamate, 10 mM L-[alpha]-aminobutyrate (an L-cysteine analog), 0.3...2, 142 mM KCl, 36 mM MgCl[sub]2, 10 mM ATP, 0.4 mM **EDTA** , various amounts of sulfoximine, and gamma-GCS. At intervals, 50 [mu]l aliquots were removed similar level of inactivation was achieved with 2 mM with [**alpha**]-ethyl-DL- **buthionine** -SR-sulfoximine (DL-SR-[alpha]-ethyl-BSO), synthesized by the method described in Griffith, O...

...but only the L-S isomer is active as an enzyme inhibitor. The concentration of [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine is [difference]1/4 of the total concentration or [difference]500 [mu]M...

...large to bind to that enzyme (see said FIG. 5 mentioned above). In separate studies, [**alpha**]-ethyl-DL- **buthionine** -SR-sulfoximine was compared to L-buthionine-S-sulfoximine as an inhibitor of mammalian gamma ...

...protocol similar to that described here for the E. coli enzyme, it was found that [**alpha**]-ethyl-DL- **buthionine** -SR-sulfoximine was 0.025% as effective as L-buthionine-S-sulfoximine as an inhibitor of the mammalian enzyme. Expressed on the basis of the active isomer, [**alpha**]-ethyl-L- **buthionine** -s-sulfoximine, [**alpha**]-ethyl-BSO has about A patient with E. coli caused gastroenteritis with bloody diarrhea is administered [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine (hereinafter [alpha]-ethyl BSO) orally at a dose of 10 mmol/kg body...
...day. Bloody diarrhea resolved over four days. Similar results obtained using the same dose of [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine given intravenously at the same dose...

Non-exemplary or Dependent Claim(s):
...7. The method of claim 6 wherein the manipulator of nitrosative stress is [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine...

8/6,KWIC/11 (Item 11 from file: 654)

4272687
Derwent Accession: 1996-497351
Utility
C/ **S-adenosyl methionine regulation of metabolic pathways and its use in diagnosis and therapy**

Fulltext Word Count: 35397
Number of Claims: 18

Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 12
Number of Figures: 12
Number of US cited patent references: 10
Number of non-US cited patent references: 2
Number of non-patent cited references: 38

Description of the Invention:
...buffer (15 mM KCl, 60 mM NaCl, 50 mM Hepes, pH 7.5, 1 mM **EDTA** , 10% glycerol; to this was added DTT to make 1 mM and a protease mix...buffer (15 mM KCl, 60 mM NaCl, 50 nM Hepes, pH 7.5, 1 mM **EDTA** , 1 mM DTT] by gentle mixing for 5 minutes followed by centrifugation for 60 second at 1000Xg. Buffer A (250 mM NaCl, 50 mM Hepes, pH 7.5, 1 mM **EDTA** , 1 mM DTT] in a volume of 0.5 ml, is added to the affinity...

...successively with Buffer B (750 mM NaCl, 50 mM Hepes, pH 7.5, 1 mM **EDTA** , 1 mM DTT) and Buffer C (7M urea, 50 mM Hepes, pH 7.5, 1 mM **EDTA** , 1 mM DTT) to yield "supernatant B" and "supernatant C". The protein concentrations of Wash...

Non-exemplary or Dependent Claim(s):
...for Pathway 1, S-adenosylinethionine (SAM), S-adenosyl homocysteine (SAH), adenosine, homocysteine, glutathione, glutathione disulfide, **cystathionine** , [**alpha**]-ketobutyrate, cysteine, cystine, taurine, choline, betaine, dimethylglycine, methylglycine, glycine, serine, folate, tetrahydrofolate, methylene tetrahydrofolate, methyltetrahydrofolate...

8/6,KWIC/12 (Item 12 from file: 654)
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4244044 **IMAGE Available
Derwent Accession: 1998-086960
Utility
C/ **Full length transcript (FLt) promoter from figwort mosaic caulimovirus (FMV) and use to express chimeric genes in plant cells**

Fulltext Word Count: 12562
Number of Claims: 12
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 17
Number of Figures: 11
Number of US cited patent references: 10
Number of non-US cited patent references: 1
Number of non-patent cited references: 18

Description of the Invention:
...extraction buffer (50 mM NaPO4, pH 7.0, 10 mM [beta]-mercaptoethanol, 10 mM Na2 **EDTA** , 0.% Na Sarkosyl, 0.1% Triton X-100), and centrifuged for 10 min. at full...extraction buffer (50 mM NaPO4, pH 7.0, 10 mM [beta]-mercaptoethanol, 10 mM Na2 **EDTA** , 0.1% Na Sarkosyl, 0.1% Triton X-100), and centrifuged for 10 min at...5 mM K3[Fe(CN)6], 0.5 mM K4[Fe(CN)6], 10 mM **EDTA** , 1 mg/ml 5-bromo-4 chloro-3-indolyl-[beta]-D-glucuronide (X-gluc) in...5. Bacterial or nematode pathogen resistance, (developed with [**alpha**]- **hordothionin** gene, Bt toxin gene, beet cyst nematode resistant locus...

8/6,KWIC/13 (Item 13 from file: 654)
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4198164
Derwent Accession: 1999-288106
Utility
C/ **Increasing the digestibility of food proteins by thioredoxin reduction**

; **FOOD TREATMENT WITH THIOREDOXIN, NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE-THIOREDOXIN REDUCTASE, REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE (NADPH)AND ADMINISTERING TREATED FOOD TO ANIMAL**

Fulltext Word Count: 35406
Number of Claims: 6
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 9
Number of Figures: 18
Number of US cited patent references: 3
Number of non-US cited patent references: 2
Number of non-patent cited references: 51

Summary of the Invention:
...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

Description of the Invention:
... **Purothionin** [**alpha**] from bread wheat and **purothionins** [**alpha**]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [**alpha**] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [**alpha**]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume ... same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** [**alpha**], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[**alpha**], failed to activate NADP-MDH (Table I) (Wada, K. et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[**alpha**] from bread wheat, two purothionins previously not examined (**purothionins** [**alpha**]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [**alpha**]-type) by the SDS-PAGE fluorescence procedure...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two [**alpha**]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...with 30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/6,KWIC/14 (Item 14 from file: 654)

4125212
Derwent Accession: 1995-269272
Utility
C/ Treatment for diabetes using a gastrin/CCK receptor ligand and an EGF receptor ligand
; ADMINISTERING GASTRIN/CHOLECYSTOKININ RECEPTOR LIGAND AND EPIDERMAL GROWTH FACTOR RECEPTOR LIGAND TO EFFECT DIFFERENTIATION OF PANCREATIC ISLET PRECURSOR CELLS TO MATURE INSULIN SECRETING CELLS

Fulltext Word Count: 5502
Number of Claims: 9
Exemplary or Independent Claim Number(s): 1,6
Number of Drawing Sheets: 5
Number of Figures: 9
Number of non-patent cited references: 4

Description of the Invention:
...NY. The TGF[alpha] transgenic line MT-42 used, which expresses high levels of TGF[**alpha**] from a **metallothionine** promoter, is described in Jhappan et al, Cell, 61:1137-1146 (1990...CsCl gradient purification, and dialyzed extensively against injection buffer (5 mM NaCl; 0.1 mM **EDTA** ; 5 mM Tris-HCl pH 7.4). Fertilized oocytes from FVB inbred mice (Taconic Farms...

8/6,KWIC/15 (Item 15 from file: 654)
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4098317
Utility
C/ **Trabecular meshwork induced glucocorticoid response (TIGR) nucleic acid molecules**
; ACCURATE GLAUCOMA DIAGNOSIS

Fulltext Word Count: 12136
Number of Claims: 6
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 4
Number of Figures: 4
Number of US cited patent references: 10
Number of non-US cited patent references: 6
Number of non-patent cited references: 32

Description of the Invention:
...other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as **EDTA** ; and sugar alcohols such as mannitol or sorbitol...pattern of induction in HTM cells was distinguishable from other steroid induced proteins such as **metallothionine** , **alpha** [sub]1 -acid glycoprotein, and TAT which were maximally induced by one day dexamethasone treatment...

8/6,KWIC/16 (Item 16 from file: 654)
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4090242
Derwent Accession: 1999-095006
Utility
C/ **Methods for the diagnosis of glaucoma**

Fulltext Word Count: 13856
Number of Claims: 10
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 4
Number of Figures: 4
Number of US cited patent references: 10
Number of non-US cited patent references: 4
Number of non-patent cited references: 30

Description of the Invention:
...other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as **EDTA** ; and sugar alcohols such as mannitol or sorbitol...pattern of induction in HTM cells was distinguishable from other steroid induced proteins such as **metallothionine** , **alpha** [sub]1 -acid glycoprotein, and TAT which were

maximally induced by one day dexamethasone treatment...

8/6,KWIC/17 (Item 17 from file: 654)

4022853
Derwent Accession: 1996-230600
Utility
C/ **Neutralization of food allergens by thioredoxin**
; DISULFIDE BONDS ARE REDUCED TO SULFHYDRYL DECREASING ALLERGENICITY OF FEEDS

Fulltext Word Count: 30535
Number of Claims: 25
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 6
Number of Figures: 6
Number of US cited patent references: 1
Number of non-patent cited references: 39

Summary of the Invention:
...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

Description of the Invention:
...Purothionin a from bread wheat and **purothionins** [**alpha**]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [**alpha**] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [**alpha**]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7 ...same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** [**alpha**], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[**alpha**], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[**alpha**] from bread wheat, two purothionins previously not examined (**purothionins** [**alpha**]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [**alpha**]-type) by the SDS-PAGE fluorescence procedure...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two [**alpha**]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...

...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/6,KWIC/18 (Item 18 from file: 654)

4019370
Derwent Accession: 1998-446069
Utility
C/ **Methods for the diagnosis of glaucoma**
; **USING A GLUCOCORTICOID INDUCED PROTEIN**

Fulltext Word Count: 18029
Number of Claims: 106
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 4
Number of Figures: 4
Number of US cited patent references: 10
Number of non-US cited patent references: 5
Number of non-patent cited references: 31

Description of the Invention:
...other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as **EDTA** ; and sugar alcohols such as mannitol or sorbitol...pattern of induction in HTM cells was distinguishable from other steroid induced proteins such as **metallothionine** , **alpha** [sub]1 -acid glycoprotein, and TAT which were maximally induced by one day dexamethasone treatment...

8/6,KWIC/19 (Item 19 from file: 654)
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4003403
Derwent Accession: 1995-090847
Utility
C/ **Antimicrobial proteins from Allium**
; **FUNGICIDES; BACTERICIDES**

Fulltext Word Count: 7927
Number of Claims: 11
Exemplary or Independent Claim Number(s): 1,2,6,8,10
Number of Drawing Sheets: 7
Number of Figures: 5
Number of non-US cited patent references: 2

Summary of the Invention:
...Rhizoctonia solani (Logemann et al, 1992, Biotechnol, 10:305-308); transgenic tobacco expressing a barley [**alpha**]- **thionin** has increased resistance to Pseudomonas bacterial pathogens (Carmona et al, 1993, Plant J, 3(3...

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mm KCl, 2 mM **EDTA** and 2 mM thiourea. After extraction, the slurry was mixed in a WARING blender and ...sample buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...filtration over a Chromaspin+TE-100 (Clontech) column equilibrated in 10 mM Tris, 1 mM **EDTA** , 300 mM NaCl, 0.05% (w/v) SDS (pH 8). RNA was subsequently removed by...

8/6,KWIC/20 (Item 20 from file: 654)
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

3985641
Derwent Accession: 1998-059387
Utility
C/ **Polyimide precursor composition, method of forming polyimide film, electronic parts and liquid crystal element**
; **COMPOSITION COMPRISING POLYAMIC ACID, CURE ACCELERATOR SELECTED FROM**

GROUP CONSISTING OF NITROGEN-CONTAINING HETEROCYCLIC COMPOUND, AMINO ACID COMPOUND, AROMATIC COMPOUND HAVING TWO OR MORE HYDROXYL GROUPS

Fulltext Word Count: 18794
Number of Claims: 20
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 5
Number of Figures: 8
Number of US cited patent references: 2
Number of non-patent cited references: 4

Description of the Invention:
...pteridine, oxazole, benzoxazole, isoxazole, benzisoxazole, thiazole, benzothiazole, isothiazole, benzisothiazole, oxadiazole, thiadiazole, pyrroledione, isoindoledione, pyrrolidinedione, benzisoquinolinedione, **triethylenediamine** , and hexamethylenetetramine. These compounds may be in the form of an N-oxide compound...isoglutamine, [alpha]-methylglutamic acid, [beta]-hydroxyglutamic acid, [gamma]-hydroxyglutamic acid, [alpha]-aminoadipic acid, citrulline, lanthionine, **cystathionine** , phenylalanine, [**alpha**]-methylphenylalanine, o-chlorophenylalanine, m-chlorophenylalanine, p-chlorophenylalanine, o-fluorophenylalanine, m-fluorophenylalanine, p-fluorophenylalanine, [beta]-(2...1,3-dione, N-hydroxypyrrolidine-2,5-dione, N-hydroxybenz de!isoquinoline-1,3-dione, **triethylenediamine** , hexamethylenetetramine, hydantoin, histidine, uracil, barbituric acid, dialuric acid, cytosine, anilinoacetic acid, N-(2-pyridyl)glycine...

Non-exemplary or Dependent Claim(s):
...cinnoline, naphthylidine, acridine, phenanthridine, benzoquinoline, benzisoquinoline, benzocinnoline, benzophthalazine, benzoquinoxaline, benzoquinazoline, phenanthroline, phenazine, carboline, perimidine, pteridine, **triethylenediamine** , and hexamethylenetetramine; or... isoglutamine, [alpha]-methylglutamic acid, [beta]-hydroxyglutamic acid, [gamma]-hydroxyglutamic acid, [alpha]-aminoadipic acid, citrulline, lanthionine, **cystathionine** , phenylalanine, [**alpha**]-methylphenylalanine, o-chlorophenylalanine, m-chlorophenylalanine, p-chlorophenylalanine, o-fluorophenylalanine, m-fluorophenylalanine, p-fluorophenylalanine, [beta]-(2...

8/6,KWIC/21 (Item 21 from file: 654)
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

3783044
Derwent Accession: 1992-150877
Utility
C/ **Directed evolution of novel binding proteins**
; GENETIC PACKAGE, CHIMERIC

Fulltext Word Count: 112846
Number of Claims: 83
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 16
Number of Figures: 16
Number of US cited patent references: 19
Number of non-US cited patent references: 10
Number of non-patent cited references: 47

Description of the Invention:
...to the binding site. For a decapeptide with three isoenergetic conformations (e.g., [beta] strand, [**alpha**] helix, and reverse turn) at each residue, there are about 6.[multiplication dot]10[sup...

8/6,KWIC/22 (Item 22 from file: 654)
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

3644278
Derwent Accession: 1992-415779
Utility
C/ **Antipathogenic peptides and compositions containing the same ; HERBICIDES**

Fulltext Word Count: 14350
Number of Claims: 8
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 1
Number of Figures: 1
Number of US cited patent references: 1
Number of non-US cited patent references: 1
Number of non-patent cited references: 8

Summary of the Invention:
...such as dithiothreitol to minimize oxidation of cystein residues, and a metal chelater such as **EDTA** to prevent exposure of the protein to heavy metal ions that might inactivate the protein...suitable non-ionic surfactants are the water-soluble adducts of polyethylene oxide with polypropylene glycol, **ethylenediamine** propylene glycol and alkylpolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain...

Description of the Invention:
...volumes of a buffer solution (80 ml) comprising 0.1 M Tris-HCl, 10 mM **EDTA** , pH 7.5...For control purposes two standards are used in the above test that is [**alpha**]1/[beta] **thionin** from wheat endosperm and LT26-thionin from barley leaves...medium is maintainedon a callus growth medium comprised of MS major, minor salts and Fe- **EDTA** (Gibco #500-1117; 4.3 g/l), MS vitamins, 100 mg/l myo-inositol, 20...

8/6,KWIC/23 (Item 1 from file: 349)

00864262
WHOLE CELL ENGINEERING BY MUTAGENIZING A SUBSTANTIAL PORTION OF A STARTING GENOME, COMBINING MUTATIONS, AND OPTIONALLY REPEATING
INGENIERIE CELLULAIRE COMPLETE PAR MUTAGENESE D'UNE PARTIE SUBSTANTIELLE D'UN GENOME DE DEPART, PAR COMBINAISON DE MUTATIONS ET EVENTUELLEMENT REPETITION
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 336587
Publication Year: 2001

Fulltext Availability:
Detailed Description

Detailed Description
... kinase
23. Aleurone 1 75. CycIodextrin glycosyItransferase
24. Alpha hordodiinonin 76. Cyfindrical inclusion protein
25. **Alpha** -arnylase 77. **Cystathionine** synthase
26. Al@ha-hemogiobin 78. Delta- 1 2 desaturase
27. Aminoglycoside 3'-adenylytransferase 79...methods, here, the interaction between biotin and avidin is overcome by employing denaturing conditions (fonnamide/ **EDTA**) to release the primer extension products of the sequencing reaction from the solid support for...

8/6,KWIC/24 (Item 2 from file: 349)

DIALOG(R)File 349:(c) 2005 WIPO/Univentio. All rts. reserv.

00144093
HIGH LEVEL INDUCIBLE EXPRESSION OF HETEROLOGOUS GENES
EXPRESSION DE GENES HETEROLOGUES POUVANT ETRE INDUITE A UN HAUT NIVEAU
Publication Language: English
Fulltext Availability:
  Detailed Description
  Claims
Fulltext Word Count: 7195
Publication Year: 1988

Fulltext Availability:
  Detailed Description
  Claims

Detailed Description
... responsive transcription units have been
identified including mouse mammary tumor virus (MMTV),
mouse and human **metallothionins** , rat **alpha** 2u-globulin, and
rat and human growth hormone, [See, e,g,, Hollenberg et al.

Nature...23M Tris@Cl pH 8.0) and the mixture
held on ice for 5 min, **EDTA** (I ml of .25 M **EDTA** pH 8.0) was
added for an additional 5 min. on ice, and then 1...

Claim
... selected from the group
consisting of the mouse mammary tumor virus LTR, mouse
metallothionine, human **metallothionine** , rat **alpha**
2u-globulin, and rat and human growth hormone
transcriptional control sequence and protions thereof which...

8/6,KWIC/25 (Item 1 from file: 340)
DIALOG(R)File 340:(c) 2005 IFI/CLAIMS(R). All rts. reserv.

2984640 9816693
C/POLYIMIDE PRECURSOR COMPOSITION, METHOD OF FORMING POLYIMIDE FILM, ELECTRONIC PARTS AND LIQUID CRYSTAL ELEMENT; COMPOSITION COMPRISING POLYAMIC ACID, CURE ACCELERATOR SELECTED FROM GROUP CONSISTING OF NITROGEN-CONTAINING HETEROCYCLIC COMPOUND, AMINO ACID COMPOUND, AROMATIC COMPOUND HAVING TWO OR MORE HYDROXYL GROUPS

Non-exemplary Claims: ...cinnoline, naphthylidine, acridine,
phenanthridine, benzoquinoline, benzisoquinoline, benzocinnoline,
benzophthalazine, benzoquinoxaline, benzoquinazoline, phenanthroline,
phenazine, carboline, perimidine, pteridine, **triethylenediamine** , and
hexamethylenetetramine; or the group consisting of pyridine, pyridazine,
pyrimidine, pyrazine, triazine, tetrazine, oxazole, benzooxazole...

...isoglutamine, Alpha -methylglutamic acid, Beta -hydroxyglutamic acid,
gamma -hydroxyglutamic acid, Alpha -aminoadipic acid, citrulline,
lanthionine, **cystathionine** , phenylalanine, **Alpha**
-methylphenylalanine, o-chlorophenylalanine, m-chlorophenylalanine,
p-chlorophenylalanine, o-fluorophenylalanine, m-fluorophenylalanine,
p-fluorophenylalanine, Beta -(2...

8/6,KWIC/26 (Item 1 from file: 348)
DIALOG(R)File 348:(c) 2005 European Patent Office. All rts. reserv.

01326487
Treatment for diabetes
Behandlung von Diabetes
Traitement du diabete
LANGUAGE (Publication,Procedural,Application): English; English; English

FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	200137	245
SPEC A	(English)	200137	5550
Total word count - document A			5795
Total word count - document B			0
Total word count - documents A + B			5795

...SPECIFICATION NY. The TGF(alpha) transgenic line MT-42 used, which expresses high levels of TGF(**alpha**) from a **metallothionine** promoter, is described in Jappan et al, Cell, 61:1137-1146 (1990).

INSGAS Transgene Construct...

...by CsCl gradient purification, and dialyzed extensively against injection buffer (5mM NaCl; 0.1 mM **EDTA** ; 5mM Tris-HCl pH 7.4). Fertilized oocytes from FVB inbred mice (Taconic Farms, Inc...

8/6,KWIC/27 (Item 2 from file: 348)

00952289

Use of thiol redox proteins for reducing protein intramolecular disulfide bonds, for improving the quality of cereal products, dough and baked goods

Verwendung von Thiol-Redox-Proteinen zur Reduktion des intramolekularen Disulfid-Bindung bei Proteinen, zur Besserung der Qualitat der Getreidenprodukte, des Te

Utilisation des proteines redox des groupes thiol pour la reduction des liaisonsdisulfide intramoleculaires, pour augmenter la qualite des produits cerealiers,

LANGUAGE (Publication,Procedural,Application): English; English; English

FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	9837	371
SPEC A	(English)	9837	25170
Total word count - document A			25541
Total word count - document B			0
Total word count - documents A + B			25541

...SPECIFICATION measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known... the E. coli NADP/Thioredoxin System.

Fig. 5 is a graph showing the effect of **purothionin** (**alpha**) and CM-1 (alpha)-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

Other proteins

Purothionin (**alpha**) from bread wheat and **purothionins** (**alpha**)-1 and (beta) from durum wheat were kind gifts from Drs. D.D. Kasarda and B.L. Jones, respectively. The **purothionin** (**alpha**) sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** (**alpha**)-1 and (beta) samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

Routine Method Steps...

...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7...5, conditions were as in Fig. 4 except that the DSG proteins were omitted and **purothionin** (**alpha**), 20 (mu)g or CM-1, 20 (mu)g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the

type tested earlier, **purothionin** -(**alpha**), failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124...

...240). However, in contrast to purothionin-a from bread wheat, two purothionins previously not examined (**purothionins** (**alpha**)-1 and (beta) from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the (**alpha**)-type) by the SDS-PAGE fluorescence procedure (Fig. 7).

EXAMPLE 6

Quantitation of Reduction

The...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two (**alpha**)-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30(degree)C for 2 hours. The concentrations of thioredoxin, NTR, and NADPH were...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below.

CM32 Chromatography

The pullulanase inhibitor sample...

...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/6,KWIC/28 (Item 3 from file: 348)

00709018

USE OF THIOL REDOX PROTEINS FOR REDUCING DISULFIDE BONDS

VERWENDUNG VON THIOLGRUPPEN ENTHALTENDEN REDOX-PROTEINEN ZUR REDUKTION VON DISULFIDBINDUNGEN

UTILISATION DE PROTEINES D'OXYDOREDUCTION A BASE DE THIOL POUR REDUIRE DES LIAISONS BISULFURES

LANGUAGE (Publication,Procedural,Application): English; English; English

FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	9901	992
CLAIMS B	(German)	9901	1020
CLAIMS B	(French)	9901	1208
SPEC B	(English)	9901	24864
Total word count - document A			0
Total word count - document B			28084
Total word count - documents A + B			28084

...SPECIFICATION measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known... the E. coli NADP/Thioredoxin System.

Fig. 5 is a graph showing the effect of **purothionin** (**alpha**) and CM-1 (alpha)-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

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and (beta) from durum wheat were kind gifts from Drs. D.D. Kasarda and B.L. Jones, respectively. The **purothionin** (**alpha**) sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** (**alpha**)-1 and (beta) samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

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EXAMPLE 6

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...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

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          $0.75  3 Types
     $1.96  Estimated cost File348
          $1.18    0.108 DialUnits File347
     $1.18  Estimated cost File347
          $0.48    0.129 DialUnits File65
     $0.48  Estimated cost File65
          $0.23    0.056 DialUnits File35
     $0.23  Estimated cost File35
          $3.89    0.219 DialUnits File342
     $3.89  Estimated cost File342
          $0.15    0.061 DialUnits File203
     $0.15  Estimated cost File203
          $0.53    0.099 DialUnits File156
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          $0.31    0.088 DialUnits File94
     $0.31  Estimated cost File94
          $8.96    0.287 DialUnits File398
     $8.96  Estimated cost File398
          $1.17    0.056 DialUnits File357
     $1.17  Estimated cost File357
          $2.61    0.246 DialUnits File73
     $2.61  Estimated cost File73
          $0.57    0.126 DialUnits File50
     $0.57  Estimated cost File50
          OneSearch, 18 files,  4.673 DialUnits FileOS
     $1.06  TELNET
    $52.41  Estimated cost this search
    $61.82  Estimated total session cost   8.223 DialUnits


### Status: Signed Off. (7 minutes)

### Status: Path 1 of [Dialog Information Services via Modem]

### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID pto-dialog)
Trying 31060000009998...Open

DIALOG INFORMATION SERVICES
PLEASE LOGON:
 ******** HHHHHHHH SSSSSSSS?
### Status: Signing onto Dialog
 ********
ENTER PASSWORD:
 ******** HHHHHHHH SSSSSSSS? ********
Welcome to DIALOG
### Status: Connected


Dialog level 04.20.00D

Reconnected in file OS 26feb05 10:12:47
* * *

SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R) 1951-2005/Feb W3
         (c) format only 2005 The Dialog Corp.
*File 155: Medline has been reloaded; acession numbers have changed.
Please see HELP NEWS 154.
  File 654:US Pat.Full. 1976-2005/Feb 24
         (c) Format only 2005 The Dialog Corp.
  File 399:CA SEARCH(R) 1967-2005/UD=14209
         (c) 2005 American Chemical Society
*File 399: Use is subject to the terms of your user/customer agreement.
Alert feature enhanced for multiple files, etc. See HELP ALERT.
  File 349:PCT FULLTEXT 1979-2002/UB=20050217,UT=20050210
         (c) 2005 WIPO/Univentio
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File 340:CLAIMS(R)/US Patent 1950-05/Feb 24

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Set     Items   Description
S1        373   ALPHA (2N) THIONIN?
S2        313   RD (unique items)
S3     279736   'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION'
S4      53755   'EDTA'
S5      44607   R1-R4
S6         79   S2 AND (S3 OR S4 OR S5 OR EDTA? OR ETHYLENEDIAMINE?)
S7         51   S6/2002:2005
S8         28   S6 NOT S7
?t s8/3,kwic/19 22 27 28 3 6 7 8 10 13 17
>>>KWIC option is not available in file(s): 398, 399
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8/3,KWIC/19 (Item 19 from file: 654)

DIALOG(R)File 654:US Pat.Full.
(c) Format only 2005 The Dialog Corp. All rts. reserv.

4003403
Derwent Accession: 1995-090847
Utility
C/ **Antimicrobial proteins from Allium**
; **FUNGICIDES; BACTERICIDES**
Inventor: Broekaert, Willem Frans, Dilbeek, BE
Cammue, Bruno Philippe Angelo, Alsemberg, BE
Rees, Sarah Bronwen, Bracknell, GB England
Assignee: Zeneca Limited(03), GB, England
Zeneca Ltd GB (Code: 32757)
Examiner: LeGuyader, John L. (Art Unit: 189)
Assistant Examiner: Larson, Thomas G.
Law Firm: Cushman Darby & Cushman IP Group of Pillsbury Madison & Sutro

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5773694	A	19980630	US 96591498	19960125
PCT	WO 9504754		19950216	WO 94GB1636	19940729
		371:	19960125		
		102e:	19960125		
Priority				GB 9316158	19930804
				GB 9317816	19930827

Fulltext Word Count: 7927

Summary of the Invention:
...Rhizoctonia solani (Logemann et al, 1992, Biotechnol, 10:305-308); transgenic tobacco expressing a barley [**alpha**]- **thionin** has increased resistance to Pseudomonas bacterial pathogens (Carmona et al, 1993, Plant J, 3(3...

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mm KCl, 2 mM **EDTA** and 2 mM thiourea. After extraction, the slurry was mixed in a WARING blender and ...sample buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...filtration over a Chromaspin+TE-100 (Clontech) column equilibrated in 10 mM Tris, 1 mM **EDTA** , 300 mM NaCl, 0.05% (w/v) SDS (pH 8). RNA was subsequently removed by...

8/3,KWIC/22 (Item 22 from file: 654)

DIALOG(R)File 654:US Pat.Full.
(c) Format only 2005 The Dialog Corp. All rts. reserv.

3644278
Derwent Accession: 1992-415779
Utility

C/ **Antipathogenic peptides and compositions containing the same ; HERBICIDES**
Inventor: Garcia-Olmedo, Francisco, Madrid, ES
Fernandez, Antonio M., Madrid, ES
Assignee: Universidad Politecnica de Madrid(03), Madrid, ES
Madrid, Universidad Politecnica de ES (Code: 36907)
Examiner: Fox, David T. (Art Unit: 183)
Assistant Examiner: McElwain, Elizabeth F.
Law Firm: Wenderoth, Lind & Ponack

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5446127	A	19950829	US 94326352	19941020
Continuation	Abandoned			US 93965284	19930125
Priority				ES 911258	19910524

Fulltext Word Count: 14350

Summary of the Invention:
...such as dithiothreitol to minimize oxidation of cystein residues, and a metal chelater such as **EDTA** to prevent exposure of the protein to heavy metal ions that might inactivate the protein...suitable non-ionic surfactants are the water-soluble adducts of polyethylene oxide with polypropylene glycol, **ethylenediamine** propylene glycol and alkylpolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain...

Description of the Invention:
...volumes of a buffer solution (80 ml) comprising 0.1 M Tris-HCl, 10 mM **EDTA** , pH 7.5...For control purposes two standards are used in the above test that is [**alpha**]1/[beta] **thionin** from wheat endosperm and LT26-thionin from barley leaves...medium is maintainedon a callus growth medium comprised of MS major, minor salts and Fe- **EDTA** (Gibco #500-1117; 4.3 g/l), MS vitamins, 100 mg/l myo-inositol, 20...

8/3,KWIC/27 (Item 2 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00952289
Use of thiol redox proteins for reducing protein intramolecular disulfide bonds, for improving the quality of cereal products, dough and baked goods
Verwendung von Thiol-Redox-Proteinen zur Reduktion des intramolekularen Disulfid-Bindung bei Proteinen, zur Besserung der Qualitat der Getreidenprodukte, des Te
Utilisation des proteines redox des groupes thiol pour la reduction des liaisonsdisulfide intramoleculaires, pour augmenter la qualite des produits cerealiers,
PATENT ASSIGNEE:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, (221079), Office of Technology Licensing, Suite 510, 2150 Shattuck Avenue, Berkeley, California 94720-1620, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;MC;NL;SE)
INVENTOR:
Buchanan, Bob, 19, Tamalpais Road, Berkeley, California 94708, (US)
Kobrehel, Karoly, Lab.de Tech.des Cereales, (INRA) 2, Place Viala, 34060 Montpellier Cedex 01, (FR)
Yee, Boihon C., 3215 Primrose Lane, Walnut Creek, California 94598, (US)
Wong, Joshua H., 9 Viewmont Terrace, South San Francisco, California 94080, (US)
Lozano, Rosa, 1155 Brighton Avenue, 16, Albany, California 94706, (US)
Jiao, Jin-an, 10555 West Flagler Street, Miami, Florida 33175, (US)
Shin, Sungho c/o Paek Kee Yoeup,, Department of Horticulture Chungbuk National Univ., Cheongju, 360-763, (KR)

LEGAL REPRESENTATIVE:
Phelip, Bruno et al (17811), c/o Cabinet Harle & Phelip 7, rue de Madrid, 75008 Paris, (FR)
PATENT (CC, No, Kind, Date): EP 863154 A1 980909 (Basic)
APPLICATION (CC, No, Date): EP 98201252 921008;
PRIORITY (CC, No, Date): US 776102 911012
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; MC; NL; SE
RELATED PARENT NUMBER(S) - PN (AN):
EP 672127 (EP 929218022)
INTERNATIONAL PATENT CLASS: C07K-014/415; C07K-014/76; A21D-002/26;
ABSTRACT WORD COUNT: 146

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	9837	371
SPEC A	(English)	9837	25170
Total word count - document A			25541
Total word count - document B			0
Total word count - documents A + B			25541

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Fig. 5 is a graph showing the effect of **purothionin** (**alpha**) and CM-1 (alpha)-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

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...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7...5, conditions were as in Fig. 4 except that the DSG proteins were omitted and **purothionin** (**alpha**), 20 (mu)g or CM-1, 20 (mu)g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -(**alpha**), failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124...

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8/3,KWIC/28 (Item 3 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00709018
USE OF THIOL REDOX PROTEINS FOR REDUCING DISULFIDE BONDS
VERWENDUNG VON THIOLGRUPPEN ENTHALTENDEN REDOX-PROTEINEN ZUR REDUKTION VON DISULFIDBINDUNGEN
UTILISATION DE PROTEINES D'OXYDOREDUCTION A BASE DE THIOL POUR REDUIRE DES LIAISONS BISULFURES
PATENT ASSIGNEE:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, (221072), 300 Lakeside Drive, 22nd Floor, Oakland, California 94612-3550, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;MC;NL;SE)
INVENTOR:
BUCHANAN, Bob, B., 19 Tamalpias Road, Berkeley, CA 94708, (US)
KOBREHEL, Karoly Laboratoire de Technologie des, Cereales (INRA) 2, place Viala, F-34060 Montpellier Cedex 01, (FR)
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WONG, Joshua, H., 9 Viewmont Terrace, South San Francisco, CA 94080, (US)
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JIAO, Jin-an, 10555 West Flagler Street, Miami, FL 33175, (US)
SHIN, Sungho Paek Kee Yoeup Department, of Horticulture Chungbuk National University, Cheongju 360-763, (KR)
LEGAL REPRESENTATIVE:
Phelip, Bruno et al (17811), c/o Cabinet Harle & Phelip 7, rue de Madrid, 75008 Paris, (FR)
PATENT (CC, No, Kind, Date): EP 672127 A1 950920 (Basic)
EP 672127 A1 960327
EP 672127 B1 990107
WO 9308274 930429
APPLICATION (CC, No, Date): EP 92921802 921008; WO 92US8595 921008
PRIORITY (CC, No, Date): US 776109 911012; US 935002 920825
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; MC; NL; SE
INTERNATIONAL PATENT CLASS: A21D-010/00; A21D-013/00; A23L-001/18; A23L-001/168; A23L-001/172; A21D-002/26;
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

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8/3,KWIC/3 (Item 3 from file: 654)

4461438 **IMAGE Available
Derwent Accession: 1996-230600

Utility

C/ **Neutralization of food allergens by thioredoxin**

; CONTACTING PROTEIN WITH AMOUNT OF THIOREDOXIN, NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE-THIOREDOXIN REDUCTASE AND NADPH OR AMOUNT OF

THIOREDOXIN AND DITHIOTHREITOL EFFECTIVE FOR DECREASING ALLERGENICITY OF PROTEIN; ADMINISTERING

Inventor: Buchanan, Bob B., Berkeley, CA
Kobrehel, Karoly, Montpellier, FR
Yee, Boihon C., Walnut Creek, CA
Lozano, Rosa, Madrid, ES
Frick, Oscar L., San Francisco, CA
Ermel, Richard W., Winters, CA
Assignee: The Regents of the University of California(02), Berkeley, CA
California, University of Regents (Code: 13234)
Examiner: Hendricks, Keith (Art Unit: 171)
Law Firm: Flehr Hohbach Test Albritton & Herbert LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6190723	A	20010220	US 9846780	19980323
Division	US 5792506	A		US 94326976	19941021
CIP	Pending			US 94211673	19940412
CIP	Abandoned			US 92935002	19920825
CIP	Abandoned			US 91776109	19911012

Fulltext Word Count: 29804

Summary of the Invention:

...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

Description of the Invention:

... **Purothionin** [**alpha**] from bread wheat and **purothionins** [**alpha**]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [**alpha**] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [**alpha**]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7...same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** [**alpha**], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[**alpha**], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[**alpha**] from bread wheat, two purothionins previously not examined (**purothionins** [**alpha**]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [**alpha**]-type) by the SDS-PAGE fluorescence procedure...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two [**alpha**]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/3,KWIC/6 (Item 6 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4413998
Derwent Accession: 1992-415779
Utility
C/ **Antipathogenic peptides and compositions containing same ; NUCLEOTIDE SEQUENCES CODING AN AMINO ACID SEQUENCE ASSOCIATED WITH THE PREVENTION OF INFECTION BY FUNGAL AND BACTERIAL PLANT PARASITES**
Inventor: Garcia-Olmedo, Francisco, Madrid, ES
Fernandez, Antonio Molina, Madrid, ES
Assignee: Universidad Politecnia de Madrid(03), Madrid, ES
Madrid, Universidad Politecnica de ES (Code: 36907)
Examiner: McElwain, Elizabeth F. (Art Unit: 168)
Law Firm: Wenderoth, Lind & Ponack, LLP.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6147281	A	20001114	US 95404607	19950315
Division	US 5446127	A		US 94326352	19941020
Continuation	Abandoned			US 93965284	19930125
Continuation	Pending			WO 92EP1130	19920521
Priority				ES 911258	19910524

Fulltext Word Count: 14201

Description of the Invention:
...such as dithiothreitol to minimize oxidation of cystein residues, and a metal chelater such as **EDTA** to prevent exposure of the protein to heavy metal ions that might inactivate the protein...suitable non-ionic surfactants are the water-soluble adducts of polyethylene oxide with polypropylene glycol, **ethylenediamine** propylene glycol and alkylpolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain...volumes of a buffer solution (80 ml) comprising 0.1 M Tris-HCl, 10 mM **EDTA** , pH 7.5...For control purposes two standards are used in the above test that is [**alpha**]1/[beta] **thionin** from wheat endosperm and LT26-thionin from barley leaves...is maintained on a callus growth medium comprised of MS major, minor salts and Fe- **EDTA** (Gibco # 500-1117; 4.3 g/l), MS vitamins, 100 mg/l myo-inositol, 20...

8/3,KWIC/7 (Item 7 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4377970
Derwent Accession: 1993-152468
Utility
C/ **Use of thiol redox proteins for reducing protein intramolecular disulfide bonds, for improving the quality of cereal products, dough and baked goods and for inactivating snake, bee and scorpion toxins**
Inventor: Buchanan, Bob B., Berkeley, CA
Kobrehel, Karoly, Montpellier, FR
Yee, Boihon C., Walnut Creek, CA
Wong, Joshua H., South San Francisco, CA
Lozano, Rosa, Madrid, ES
Jiao, Jin-an, Miami, FL
Shin, Sungho, Taejon, KR South Korea
Assignee: The Regents of the University of California(02), Oakland, CA
California, University of Regents (Code: 13234)
Examiner: Sisson, Bradley (Art Unit: 163)
Assistant Examiner: Bugaisky, Gabriele E.

Combined Principal Attorneys: Smith, Karen S.Flehr`Hohbach Test Albritton & Herbert LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6114504	A	20000905	US 95483930	19950607
Division	Pending			US 211673	

Fulltext Word Count: 26012

Summary of the Invention:

...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

Description of the Drawings:

...FIG. 5 is a graph showing the effect of **purothionin** [**alpha**] and CM-1 [alpha]-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...

Description of the Invention:

...Purothionin a from bread wheat and **purothionins** [**alpha**]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [**alpha**] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [**alpha**]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7 ...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[**alpha**], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124: 237-240). However, in contrast to **purothionin** -[**alpha**] from bread wheat, two purothionins previously not examined (**purothionins** [**alpha**]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [**alpha**]-type) by the SDS-PAGE fluorescence procedure (FIG. 7...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two [**alpha**]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...mM Tris-HC l, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/3,KWIC/8 (Item 8 from file: 654)

DIALOG(R)File 654:US Pat.Full.

4377417

Derwent Accession: 1993-152468

Utility

REASSIGNED

C/ Use of thiol redox proteins for reducing protein intramolecular disulfide bonds, for improving the quality of cereal products, dough and

baked goods and for inactivating snake, bee and scorpion toxins

Inventor: Buchanan, Bob B., Berkeley, CA
Kobrehel, Karoly, Montpellier, FR
Yee, Boihon C., Walnut Creek, CA
Wong, Joshua H., South San Francisco, CA
Lozano, Rosa, Madrid, ES
Jiao, Jin-an, Miami, FL
Shin, Sungho, Taejon, KR South Korea
Assignee: The Regents of the University of California(02), CA
California, University of Regents (Code: 13234)
Examiner: Sisson, Bradley (Art Unit: 163)
Assistant Examiner: Bugaisky, Gabriele E.
Combined Principal Attorneys: Smith, Karen S.Flehr Hohbach Test Albritton & Herbert LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6113951	A	20000905	US 94211673	19941121
CIP	Abandoned			US 92935002	19920825
CIP	Abandoned			US 91776109	19911012
PCT	WO 9308274		19930429	WO 92US8595	19921008
			371:19941121		
			102e:19941121		

Fulltext Word Count: 28198

Summary of the Invention:

...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area,

Description of the Drawings:

...FIG. 5 is a graph showing the effect of **purothionin** [**alpha**] and CM-1 [alpha]-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...

Description of the Invention:

... **Purothionin** [**alpha**] from bread wheat and **purothionins** [**alpha**]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [**alpha**] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [**alpha**]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...reaction was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing mM **EDTA** and 16% glycerol in a final volume of ...5, conditions were as in FIG. 4 except that the DSG proteins were omitted and **purothionin** [**alpha**], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[**alpha**], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[**alpha**] from bread wheat, two purothionins previously not examined (**purothionins** [**alpha**]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [**alpha**]-type) by the SDS-PAGE fluorescence procedure (FIG. 7...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two [**alpha**]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant,

EDTA and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/3,KWIC/10 (Item 10 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4314235
Derwent Accession: 1998-216927
Utility
CERTIFICATE OF CORRECTION
C/ **Manipulating nitrosative stress to kill pathologic microbes, pathologic helminths and pathologically proliferating cells or to upregulate nitrosative stress defenses**
Inventor: Stamler, Jonathan S., Chapel Hill, NC
Griffith, Owen W., Milwaukee, WI
Assignee: Duke University(02), Durham, NC
The Medical College of Wisconsin Research Foundation, Inc.(02), Milwaukee, WI
Duke University
Medical College of Wisconsin The (Code: 05187 25202)
Examiner: Weddington, Kevin E. (Art Unit: 164)

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6057367	A	20000502	US 97852490	19970507
Provisional				US 60-25819	19960830

Fulltext Word Count: 30970

Summary of the Invention:
...alkyl contains 1 to 10 carbon atoms. Some examples of species of this genus are [**alpha**]-ethyl- **buthionine** sulfoximine, [**alpha**]-propyl- **buthionine** sulfoximine, [**alpha**]-isopropyl- **buthionine** sulfoximine, and [**alpha**]-tert butyl- **buthionine** sulfoximine

Description of the Invention:
...A preferred selective inhibitor of glutathione synthesis within the above-described genus is [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine, which can be readily prepared by the method described above, i.e., by...

...and sodium cyanide to form the corresponding hydantoin, hydrolyzing the hydantoin in alkali to form [**alpha**]-ethyl-DL- **buthionine** , converting that compound to the corresponding sulfoximine by reaction with sodium azide in sulfuric acidOther sulfoximines embraced by the genus described above, include, for example, [**alpha**]-propyl-DL- **buthionine** -SR-sulfoximine, [**alpha**]-isopropyl-DL- **buthionine** -SR-sulfoximine, [**alpha**]-butyl-DL- **buthionine** -SR-sulfoximine, [**alpha**]-tert butyl-DL- **buthionine** -SR-sulfoxime, [**alpha**]-ethyl-S-butyl-[delta]-thionorvaline sulfoximine, and [alpha]-propyl-S-butyl-[delta]-thionorvaline sulfoximine. Also...where the [alpha]-alkyl is ethyl and/or the S-alkyl is butyl, e.g., [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine, with the dosages and routes of administration described above for these applying here...mM MgCl[sub]2, 7.5 mM ATP, 7.5 mM phosphoenolpyruvate, 0.3 mM **EDTA** , 10 mM L-glutamate, 10 mM L-[alpha]-aminobutyrate (an L-cysteine analog), 0.3...2, 142 mM KCl, 36 mM MgCl[sub]2, 10 mM ATP, 0.4 mM **EDTA** , various amounts of sulfoximine, and gamma-GCS. At intervals, 50 [mu]l aliquots were removed similar level of inactivation was achieved with 2 mM with [**alpha**]-ethyl-DL- **buthionine** -SR-sulfoximine

(DL-SR-[alpha]-ethyl-BSO), synthesized by the method described in Griffith, O...

...but only the L-S isomer is active as an enzyme inhibitor. The concentration of [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine is [difference]1/4 of the total concentration or [difference]500 [mu]M...

...large to bind to that enzyme (see said FIG. 5 mentioned above). In separate studies, [**alpha**]-ethyl-DL- **buthionine** -SR-sulfoximine was compared to L-buthionine-S-sulfoximine as an inhibitor of mammalian gamma ...

...protocol similar to that described here for the E. coli enzyme, it was found that [**alpha**]-ethyl-DL- **buthionine** -SR-sulfoximine was 0.025% as effective as L-buthionine-S-sulfoximine as an inhibitor of the mammalian enzyme. Expressed on the basis of the active isomer, [**alpha**]-ethyl-L- **buthionine** -s-sulfoximine, [**alpha**]-ethyl-BSO has about A patient with E. coli caused gastroenteritis with bloody diarrhea is administered [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine (hereinafter [alpha]-ethyl BSO) orally at a dose of 10 mmol/kg body...

...day. Bloody diarrhea resolved over four days. Similar results obtained using the same dose of [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine given intravenously at the same dose...

Non-exemplary or Dependent Claim(s):
...7. The method of claim 6 wherein the manipulator of nitrosative stress is [**alpha**]-ethyl-L- **buthionine** -S-sulfoximine...

8/3,KWIC/13 (Item 13 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4198164
Derwent Accession: 1999-288106
Utility
C/ **Increasing the digestibility of food proteins by thioredoxin reduction ; FOOD TREATMENT WITH THIOREDOXIN, NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE-THIOREDOXIN REDUCTASE, REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE (NADPH)AND ADMINISTERING TREATED FOOD TO ANIMAL**
Inventor: Buchanan, Bob B., Berkeley, CA
del Val, Gregorio, Saint-Aubin/NE, CH
Lozano, Rosa M., Madrid, ES
Jiao, Jin-an, Ft. Lauderdale, FL
Wong, Joshua H., South San Francisco, CA
Yee, Boihon C., Walnut Creek, CA
Assignee: The Regents of the University of California(02), Oakland, CA
California, University of Regents (Code: 13234)
Examiner: Hendricks, Keith D. (Art Unit: 171)
Combined Principal Attorneys: Smith, Karen S.Flehr Hohbach Test Albritton & Herbert LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5952034	A	19990914	US 97953703	19971017
CIP	US 5792506	A		US 94326976	19941021
CIP	Pending			US 94211673	19940412
CIP	Abandoned			US 92935002	19920825
CIP	Abandoned			US 91776109	19911012

Fulltext Word Count: 35406

Summary of the Invention:
...measures are taken to minimize shock, renal failure and respitory

failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

Description of the Invention:

... **Purothionin** [**alpha**] from bread wheat and **purothionins** [**alpha**]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [**alpha**] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [**alpha**]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume ... same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** [**alpha**], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[**alpha**], failed to activate NADP-MDH (Table I) (Wada, K. et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[**alpha**] from bread wheat, two purothionins previously not examined (**purothionins** [**alpha**]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [**alpha**]-type) by the SDS-PAGE fluorescence procedure...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two [**alpha**]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...with 30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/3,KWIC/17 (Item 17 from file: 654)

4022853
Derwent Accession: 1996-230600
Utility
REASSIGNED
C/ **Neutralization of food allergens by thioredoxin**
; **DISULFIDE BONDS ARE REDUCED TO SULFHYDRYL DECREASING ALLERGENICITY OF FEEDS**

Inventor: Buchanan, Bob B., Berkeley, CA
Kobrehel, Karoly, Montpellier, FR
Yee, Boihon C., Walnut Creek, CA
Lozano, Rosa, Madrid, ES
Frick, Oscar L., San Francisco, CA
Ermel, Richard W., Winters, CA
Assignee: The Regents of the University of California(02), Oakland, CA
California, University of Regents (Code: 13234)
Examiner: Grimes, Eric (Art Unit: 184)
Combined Principal Attorneys: Smith, Karen S.Flehr Hohbach Test Albritton & Herbert LLP

Publication Number	Kind	Date	Application Number	Filing Date

Main Patent	US 5792506	A	19980811	US 94326976	19941021
CIP	Pending			US 94211673	19940412
CIP	Abandoned			US 92935002	19920825
CIP	Abandoned			US 91776109	19911012

Fulltext Word Count: 30535

Summary of the Invention:

...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

Description of the Invention:

...Purothionin a from bread wheat and **purothionins** [**alpha**]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [**alpha**] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [**alpha**]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7 ...same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** [**alpha**], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[**alpha**], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[**alpha**] from bread wheat, two purothionins previously not examined (**purothionins** [**alpha**]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [**alpha**]-type) by the SDS-PAGE fluorescence procedure...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two [**alpha**]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...

...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

?e purothionin

Ref	Items	Index-term
E1	1	PUROTHIONEIN
E2	1	PUROTHIONIM
E3	525	*PUROTHIONIN
E4	1	PUROTHIONIN (RYE GENE PUR-R1 PRECURSOR REDUCED
E5	1	PUROTHIONIN (WHEAT GENE PUR-A1 PRECURSOR REDUC
E6	1	PUROTHIONIN (WHEAT GENE PUR-B1 PRECURSOR REDUC
E7	1	PUROTHIONIN (WHEAT GENE PUR-D1 PRECURSOR REDUC
E8	4	PUROTHIONIN A I
E9	16	PUROTHIONIN A I(REDUCED)
E10	1	PUROTHIONIN A II
E11	1	PUROTHIONIN A II(REDUCED)
E12	1	PUROTHIONIN CONTENT

Enter P or PAGE for more

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?p

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E13        2  PUROTHIONIN DERIVATIVE
E14        1  PUROTHIONIN HOMOLOG II (BARLEY ENDOSPERM REDUC
E15        1  PUROTHIONIN PROTEIN-PROTEIN INTERACTION
E16        2  PUROTHIONIN-ALPHA
E17        1  PUROTHIONIN-BETA
E18        1  PUROTHIONIN,.ALPHA.-
E19        1  PUROTHIONIN,.BETA.-
E20        1  PUROTHIONIN,HYDROCHLORIDE
E21       24  PUROTHIONINE
E22        1  PUROTHIONINELUTED
E23        5  PUROTHIONINES
E24        1  PUROTHIONINLIKE

           Enter P or PAGE for more
?p

Ref    Items  Index-term
E25        1  PUROTHIONINOX
E26        1  PUROTHIONINRD
E27      295  PUROTHIONINS
E28        5  PUROTHIONINS,.ALPHA.-
E29       10  PUROTHIONINS,.ALPHA.-HORDOTHIONINS
E30       11  PUROTHIONINS,.ALPHA.1-
E31        3  PUROTHIONINS,.ALPHA.2-
E32       16  PUROTHIONINS,.BETA.-
E33        3  PUROTHIONINS,.BETA.-HORDOTHIONINS
E34        3  PUROTHIONINS,.GAMMA.1-
E35        1  PUROTHIONINS,.GAMMA.2-
E36        3  PUROTHIONINS,AVENOTHIONINS

           Enter P or PAGE for more
?s e3-e33
              525  PUROTHIONIN
                1  PUROTHIONIN (RYE GENE PUR-R1 PRECURSOR REDUCED
                1  PUROTHIONIN (WHEAT GENE PUR-A1 PRECURSOR REDUC
                1  PUROTHIONIN (WHEAT GENE PUR-B1 PRECURSOR REDUC
                1  PUROTHIONIN (WHEAT GENE PUR-D1 PRECURSOR REDUC
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               16  PUROTHIONIN A I(REDUCED)
                1  PUROTHIONIN A II
                1  PUROTHIONIN A II(REDUCED)
                1  PUROTHIONIN CONTENT
                2  PUROTHIONIN DERIVATIVE
                1  PUROTHIONIN HOMOLOG II (BARLEY ENDOSPERM REDUC
                1  PUROTHIONIN PROTEIN-PROTEIN INTERACTION
                2  PUROTHIONIN-ALPHA
                1  PUROTHIONIN-BETA
                1  PUROTHIONIN,.ALPHA.-
                1  PUROTHIONIN,.BETA.-
                1  PUROTHIONIN,HYDROCHLORIDE
               24  PUROTHIONINE
                1  PUROTHIONINELUTED
                5  PUROTHIONINES
                1  PUROTHIONINLIKE
                1  PUROTHIONINOX
                1  PUROTHIONINRD
              295  PUROTHIONINS
                5  PUROTHIONINS,.ALPHA.-
               10  PUROTHIONINS,.ALPHA.-HORDOTHIONINS
               11  PUROTHIONINS,.ALPHA.1-
                3  PUROTHIONINS,.ALPHA.2-
               16  PUROTHIONINS,.BETA.-
                3  PUROTHIONINS,.BETA.-HORDOTHIONINS
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?p

Ref    Items  Index-term
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E38       14  PUROTHIONINS,HORDOTHIONINS
E39        1  PUROTHIONINS,SECALETHIONINS
E40        1  PUROTHIONIRL
E41        1  PUROTHIONNIN
E42        1  PUROTHIORINS
E43        2  PUROTI
E44        1  PUROTID
E45       15  PUROTIE
E46        1  PUROTIONIN
E47        1  PUROTNYCIN
E48        1  PUROTOC

          Enter P or PAGE for more
?s e42
     S10       1  'PUROTHIORINS'
?ds

Set     Items   Description
S1        373   ALPHA (2N) THIONIN?
S2        313   RD (unique items)
S3     279736   'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION'
S4      53755   'EDTA'
S5      44607   R1-R4
S6         79   S2 AND (S3 OR S4 OR S5 OR EDTA? OR ETHYLENEDIAMINE?)
S7         51   S6/2002:2005
S8         28   S6 NOT S7
S9        689   E3-E33
S10         1   'PUROTHIORINS'
?s s9 or s10
              689  S9
                1  S10
     S11      689  S9 OR S10
?s s11 and (edta? or ethylene? or ethylenediamine? or edta)
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>>>File 399 processing for ETHYLENE? stopped at ETHYLENEDIAMINIUM
>>>File 349 processing for ETHYLENE? stopped at ETHYLENEFLUOROPOLYMER
>>>File 340 processing for ETHYLENE? stopped at ETHYLENESULFINYL
>>>File 348 processing for ETHYLENE? stopped at ETHYLENELAURYLAMINEETHER
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           280289  EDTA?
          1981588  ETHYLENE?
           198685  ETHYLENEDIAMINE?
           279736  EDTA
     S12      128  S11 AND (EDTA? OR ETHYLENE? OR ETHYLENEDIAMINE? OR EDTA)
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>>>  or undefined in one or more files.
>>>Year ranges not supported in one or more files
              128  S12
         15341873  PY=2002 : PY=2005
     S13       50  S12/2002:2005
?s s12 not s13
              128  S12
               50  S13
     S14       78  S12 NOT S13
?ds

Set     Items   Description
S1        373   ALPHA (2N) THIONIN?
S2        313   RD (unique items)
S3     279736   'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION'
S4      53755   'EDTA'
S5      44607   R1-R4
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S6          79   S2 AND (S3 OR S4 OR S5 OR EDTA? OR ETHYLENEDIAMINE?)
S7          51   S6/2002:2005
S8          28   S6 NOT S7
S9         689   E3-E33
S10          1   'PUROTHIORINS'
S11        689   S9 OR S10
S12        128   S11 AND (EDTA? OR ETHYLENE? OR ETHYLENEDIAMINE? OR EDTA)
S13         50   S12/2002:2005
S14         78   S12 NOT S13
?s s14 not s8
               78  S14
               28  S8
     S15       70  S14 NOT S8
?t s15/6/all
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15/6/1 (Item 1 from file: 155)
08856644 PMID: 2610351
Histochemical localization of cysteine-rich proteins by tissue printing on nitrocellulose.
Nov 1 1989

15/6/2 (Item 1 from file: 654)
0004925643
Derwent Accession: 2002-010726
Compositions and methods for identifying and targeting cancer cells of alimentary canal origin

Fulltext Word Count: 30220
Number of Claims: 38
Exemplary or Independent Claim Number(s): 1,11,21,25,29,33,34,37

15/6/3 (Item 2 from file: 654)
0004918035
Derwent Accession: 2002-381264
Compositions and methods for identifying and targeting cancer cells of alimentary canal origin

Fulltext Word Count: 30022
Number of Claims: 38
Exemplary or Independent Claim Number(s): 1,11,21,25,29,33,34,37

15/6/4 (Item 3 from file: 654)
0004903757 **IMAGE Available
Derwent Accession: 1993-100978
Biocidal proteins

Fulltext Word Count: 14428
Number of Claims: 43
Exemplary or Independent Claim Number(s):
1,6,7,8,9,10,11,12,13,14,15,16,17,18,20,25,36,37
Number of Drawing Sheets: 42
Number of Figures: 42

15/6/5 (Item 4 from file: 654)
4547046
Derwent Accession: 1995-178646
Utility
C/ Imaging of colorectal cancer using ST receptor binding compounds ; VISUALIZING TUMOR CELLS; ADMINISTER RADIOACTIVE PARTICLES TO HUMANS AND DETECT POSITIONING AND ACCUMULATION OF RADIOACTIVE PARTICLES IN BODY

Fulltext Word Count: 28331
Number of Claims: 17

Exemplary or Independent Claim Number(s): 11
Number of US cited patent references: 10
Number of non-US cited patent references: 1
Number of non-patent cited references: 70

15/6/6 (Item 5 from file: 654)
4458415 **IMAGE Available
Derwent Accession: 1993-100978
Utility
C/ **Biocidal proteins**
; AMINO ACID SEQUENCES OF MICROBIOCIDAL PROTEINS; FOR TRANSGENIC PLANTS WITH INCREASED DISEASE RESISTANCE

Fulltext Word Count: 11346
Number of Claims: 2
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 39
Number of Figures: 60
Number of US cited patent references: 4
Number of non-US cited patent references: 2
Number of non-patent cited references: 15

15/6/7 (Item 6 from file: 654)
4458048
Derwent Accession: 1998-456873
Utility
C/ **Methods of identifying and detecting pancreatic cancer**
; IN VITRO DIAGNOSIS OF TUMORS IN HUMANS; ANALYZING PREFERENTIAL ORGAN TISSUE FOR PRESENCE OF CHOLECYSTOKININ MESSENGER RIBONUCLEIC ACIDS, PRESENCE OF CHOLECYSTOKININ MESSENGER RIBONUCLEIC ACIDS INDICATES HUMAN HAS A TUMOR

Fulltext Word Count: 23168
Number of Claims: 27
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 55
Number of non-patent cited references: 35

15/6/8 (Item 7 from file: 654)
4427576
Derwent Accession: 2000-687452
Utility
C/ **X-ray guided drug delivery**
; TARGETING A TISSUE; EXPOSURE OF TISSUE TO IONIZING RADIATION

Fulltext Word Count: 26146
Number of Claims: 104
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 9
Number of non-US cited patent references: 1
Number of non-patent cited references: 4

15/6/9 (Item 8 from file: 654)
4384858
Derwent Accession: 1999-180474
Utility
C/ **Compositions that specifically bind to colorectal cancer cells and methods of using the same**
; IN VITRO METHOD OF DIAGNOSING METASTASIZED COLORECTAL CANCER BY DETECTING GENE EXPRESSION OF COLORECTAL CANCER-ASSOCIATED TRANSCRIPT-1, AN ALTERNATE FORM OF HEAT-STABLE TOXIN RECEPTOR, IN CELLS OF SAMPLE

Fulltext Word Count: 34307

Number of Claims: 11
Exemplary or Independent Claim Number(s): 7
Number of Drawing Sheets: 1
Number of Figures: 1
Number of US cited patent references: 49
Number of non-patent cited references: 64

15/6/10 (Item 9 from file: 654)
4317418
Derwent Accession: 1995-178646
Utility
C/ **Compositions that specifically bind to colorectal cancer cells and methods of using the same**
; CONJUGATED COMPOUNDS WHICH COMPRISE AN ST RECEPTOR BINDING MOIETY AND A RADIOSTABLE ACTIVE MOIETY

Fulltext Word Count: 39301
Number of Claims: 10
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 12
Number of non-US cited patent references: 1
Number of non-patent cited references: 76

15/6/11 (Item 10 from file: 654)
4239629
Derwent Accession: 1994-249225
Utility
C/ **High lysine derivatives of [alpha]-hordothionin**
; FOR TRANSFORMED PLANTS AND CELLS WITH ENHANCED LYSINE CONTENT AND WHICH SUPPRESS AND KILL PLANT PATHOGENS INCLUDING FUNGI

Fulltext Word Count: 4619
Number of Claims: 21
Exemplary or Independent Claim Number(s): 1,4,6,7,10
Number of Drawing Sheets: 2
Number of Figures: 4
Number of US cited patent references: 1
Number of non-US cited patent references: 9
Number of non-patent cited references: 14

15/6/12 (Item 11 from file: 654)
4235015
Derwent Accession: 1994-183512
Utility
C/ **Transgenic plants expressing biocidal proteins**
; ISOLATED ANTIMICROBIAL PROTEIN HAVING AMINO ACID SEQUENCECONTAINING COMMON CYSTEINE/GLYCINE DOMAIN OF CHITIN BINDINGPLANT PROTEINS; AGICULTURAL AND PHARMACEUTICAL APPLICATIONS AS FUNGICIDES OR BACETRICIDES

Fulltext Word Count: 13574
Number of Claims: 20
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 17
Number of Figures: 29
Number of US cited patent references: 8
Number of non-patent cited references: 7

15/6/13 (Item 12 from file: 654)
4187951
Derwent Accession: 1992-331736
Utility
C/ **Biocidal proteins**
; TRANSFORMED BIOLOGICAL PROTEIN

Fulltext Word Count: 6005
Number of Claims: 3
Exemplary or Independent Claim Number(s): 1,3
Number of Drawing Sheets: 11
Number of Figures: 12
Number of US cited patent references: 3
Number of non-US cited patent references: 1
Number of non-patent cited references: 3

15/6/14 (Item 13 from file: 654)
4163914
Derwent Accession: 1995-246394
Utility
C/ **Transformed plants expressing antimicrobial proteins**
; ANTIMICROBIAL PROTEIN CAPABLE OF ISOLATION FROM SEEDS OF HEUCHERA OR AESCULUS

Fulltext Word Count: 5637
Number of Claims: 8
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 5
Number of Figures: 9
Number of non-US cited patent references: 2
Number of non-patent cited references: 7

15/6/15 (Item 14 from file: 654)
4118486
Derwent Accession: 1995-178646
Utility
C/ **Methods of treating metastatic colorectal cancer with ST receptor binding compounds**
; RADIOACTIVE THERAPEUTIC AGENT, RECEPTOR BINDING MOIETY

Fulltext Word Count: 33695
Number of Claims: 58
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 27
Number of non-US cited patent references: 1
Number of non-patent cited references: 83

15/6/16 (Item 15 from file: 654)
4085182
Derwent Accession: 1997-480228
Utility
C/ **Alteration of amino acid compositions in seeds**

Fulltext Word Count: 12377
Number of Claims: 28
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 6
Number of Figures: 6
Number of US cited patent references: 1
Number of non-US cited patent references: 3
Number of non-patent cited references: 33

15/6/17 (Item 16 from file: 654)
4057665
Derwent Accession: 1993-100978
Utility
C/ **Biocidal proteins**
; ANTIFUNGAL, ISOLATED FROM PLANT SEEDS

Fulltext Word Count: 11956
Number of Claims: 19
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 39
Number of Figures: 42
Number of US cited patent references: 2
Number of non-US cited patent references: 2
Number of non-patent cited references: 13

15/6/18 (Item 17 from file: 654)
3992551
Derwent Accession: 1996-188141
Utility
C/ **Methods for the identification of compounds capable of inducing the nuclear translocation of a receptor complex comprising the glucocoticoid receptor type II and viral protein R interacting protein**
; DETECTING THE HUMAN IMMUNODEFICIENCY VIRUS TYPE I PROTEIN

Fulltext Word Count: 15683
Number of Claims: 5
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 2
Number of non-patent cited references: 46

15/6/19 (Item 18 from file: 654)
3979152
Derwent Accession: 1995-246394
Utility
C/ **Antimicrobial proteins**
; PLANT EXTRACTS

Fulltext Word Count: 5652
Number of Claims: 5
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 5
Number of Figures: 5
Number of non-US cited patent references: 2
Number of non-patent cited references: 1

15/6/20 (Item 19 from file: 654)
3913150
Derwent Accession: 1994-183512
Utility
C/ **DNA encoding biocidal proteins**
; DNA SEQUENCES IN PROTEINS AND VECTORS IN CELLS

Fulltext Word Count: 14086
Number of Claims: 60
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 17
Number of Figures: 29
Number of US cited patent references: 3
Number of non-patent cited references: 6

15/6/21 (Item 20 from file: 654)
3910716 **IMAGE Available
Derwent Accession: 1992-331736
Utility
C/ **Biocidal proteins**
; GENETIC ENGINEERING

Fulltext Word Count: 6067
Number of Claims: 8

Exemplary or Independent Claim Number(s): 1,6
Number of Drawing Sheets: 11
Number of Figures: 22
Number of US cited patent references: 3
Number of non-US cited patent references: 1
Number of non-patent cited references: 3

15/6/22 (Item 21 from file: 654)
3910711
Derwent Accession: 1993-100978
Utility
C/ **Biocidal proteins**
; **GENETIC ENGINEERING**

Fulltext Word Count: 11738
Number of Claims: 13
Exemplary or Independent Claim Number(s): 1,9
Number of Drawing Sheets: 39
Number of Figures: 44
Number of US cited patent references: 2
Number of non-US cited patent references: 2
Number of non-patent cited references: 12

15/6/23 (Item 22 from file: 654)
3811293
Derwent Accession: 1994-183512
Utility
C/ **Biocidal proteins**
; **BACTERICIDES, FUNGICIDES**

Fulltext Word Count: 14080
Number of Claims: 25
Exemplary or Independent Claim Number(s): 3
Number of Drawing Sheets: 17
Number of Figures: 29
Number of US cited patent references: 4
Number of non-patent cited references: 6

15/6/24 (Item 23 from file: 654)
3746704
Derwent Accession: 1993-100978
Utility
CM/ **Biocidal proteins**
; **ANTIFUNGAL OR ANTIBACTERIAL AGENTS**

Fulltext Word Count: 11737
Number of Claims: 14
Exemplary or Independent Claim Number(s): 1,14
Number of Drawing Sheets: 39
Number of Figures: 42
Number of US cited patent references: 2
Number of non-US cited patent references: 2
Number of non-patent cited references: 13

15/6/25 (Item 24 from file: 654)
3725121
Derwent Accession: 1995-178646
Utility
C/ **ST receptor binding compounds and methods of using the same**
; **IMAGING METASTASIZED COLORECTAL CANCER, TOXTIN PEPTIDES LESS THAN 25 UNITS**

Fulltext Word Count: 28768

Number of Claims: 22
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 1
Number of non-patent cited references: 51

15/6/26 (Item 25 from file: 654)
3720578
Derwent Accession: 1994-183512
Utility
C/ **Biocidal proteins from plants**
; CHITINASES, FUNGICIDES

Fulltext Word Count: 13401
Number of Claims: 12
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 17
Number of Figures: 29
Number of US cited patent references: 3
Number of non-patent cited references: 6

15/6/27 (Item 26 from file: 654)
3685299 **IMAGE Available
Derwent Accession: 1992-331736
Utility
C/ **Biocidal proteins**

Fulltext Word Count: 6028
Number of Claims: 5
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 11
Number of Figures: 22
Number of US cited patent references: 2
Number of non-US cited patent references: 1
Number of non-patent cited references: 3

15/6/28 (Item 27 from file: 654)
3181895
Derwent Accession: 1989-220454
Utility
C/ **Use of thioredoxin, thioredoxin-derived, or thioredoxin-like dithiol peptides in hair care preparations**

Fulltext Word Count: 3722
Number of Claims: 11
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 6

15/6/29 (Item 28 from file: 654)
3045751
Derwent Accession: 1987-158828
Utility
C/ **Thioredoxin shufflease and use thereof**

Fulltext Word Count: 6059
Number of Claims: 10
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 1
Number of non-patent cited references: 4

15/6/30 (Item 29 from file: 654)
3034492
Derwent Accession: 1989-220454

Utility
C/ Use of thioredoxin, thioredoxin-derived, or thioredoxin-like dithiol peptides in hair care preparations
; SYNERGISTIC MIXTURE WITH SULFITES AND BISULFITES APPLIED

Fulltext Word Count: 3257
Number of Claims: 1
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 1
Number of non-US cited patent references: 2
Number of non-patent cited references: 1

15/6/31 (Item 30 from file: 654)
2902171
Derwent Accession: 1987-258442
Utility
C/ Method and ophthalmic composition for the prevention and reversal of cataracts
; TOPICAL ADMINISTRATION OF THIOREDOXIN

Fulltext Word Count: 3845
Number of Claims: 8
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 1
Number of non-patent cited references: 14

15/6/32 (Item 1 from file: 349)
00851452
PRODUCTION AND USE OF PROTEIN VARIANTS HAVING MODIFIED IMMUNOGENECITY
VARIANTS DE PROTEINES A IMMUNOGENICITE MODIFIEE
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 146189
Publication Year: 2001

15/6/33 (Item 2 from file: 349)
00840858
COMPOSITIONS AND METHODS FOR IDENTIFYING AND TARGETING CANCER CELLS
COMPOSITIONS ET PROCEDES D'IDENTIFICATION ET DE CIBLAGE DE CELLULES CANCEREUSES
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 37104
Publication Year: 2001

15/6/34 (Item 3 from file: 349)
00840857
COMPOSITIONS AND METHODS FOR IDENTIFYING AND TARGETING CANCER CELLS OF ALIMENTARY CANAL ORIGIN
COMPOSITIONS ET PROCEDES D'IDENTIFICATION ET DE CIBLAGE DE CELLULES CANCEREUSES PROVENANT DU TUBE DIGESTIF
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 27589

Publication Year: 2001

15/6/35 (Item 4 from file: 349)
00830501
MEMBRANE ESTROGEN RECEPTOR-DIRECTED THERAPY IN BREAST CANCER
THERAPIE DIRIGEE SUR LE RECEPTEUR MEMBRANAIRE DES OESTROGENES, DANS LE CANCER DU SEIN
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 28454
Publication Year: 2001

15/6/36 (Item 5 from file: 349)
00784061
NUCLEIC ACID SEQUENCES ENCODING CELL WALL-DEGRADING ENZYMES AND USE TO ENGINEER RESISTANCE TO FUSARIUM AND OTHER PATHOGENS
SEQUENCES D'ACIDE NUCLEIQUE CODANT POUR DES ENZYMES DEGRADANT LES PAROIS CELLULAIRES ET LEUR UTILISATION POUR CREER UNE RESISTANCE AU FUSARIUM ET A D'AUTRES PATHOGENES
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 40894
Publication Year: 2001

15/6/37 (Item 6 from file: 349)
00754251
X-RAY GUIDED DRUG DELIVERY
ADMINISTRATION DE MEDICAMENT GUIDEE PAR RAYON X
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 41390
Publication Year: 2000

15/6/38 (Item 7 from file: 349)
00746101
BARLEY GENE FOR THIOREDOXIN AND NADP-THIOREDOXIN REDUCTASE
GENE D'ORGE POUR REDUCTASE DE THIOREDOXINE ET DE THIOREDOXINE NADP
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 51098
Publication Year: 2000

15/6/39 (Item 8 from file: 349)
00745163 **Image available**
PLANTS TRANSFORMED WITH THIOREDOXIN
VALORISATION DE GRAINES ET DE SEMENCES TRANSFORMEES PAR THIOREDOXINE
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description

Claims
Fulltext Word Count: 34481
Publication Year: 2000

15/6/40 (Item 9 from file: 349)
00731623 **Image available**
ALLEVIATION OF THE ALLERGENIC POTENTIAL OF AIRBORNE AND CONTACT ALLERGENS BY THIOREDOXIN
DIMINUTION DU POTENTIEL ALLERGENIQUE D'ALLERGENES PORTES PAR L'AIR OU AGISSANT PAR CONTACT A L'AIDE DE THIOREDOXINE
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 44560
Publication Year: 2000

15/6/41 (Item 10 from file: 349)
00577322
SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED THEREBY
FRAGMENTS D'ADN DETERMINES SELON LEUR SEQUENCE ET POLYPEPTIDES CORRESPONDANTS CODES PAR LESDITS FRAGMENTS
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 226130
Publication Year: 2000

15/6/42 (Item 11 from file: 349)
00517693 **Image available**
RECOMBINANT MAJOR ALLERGEN OF THE POLLEN OF ARTEMISIA VULGARIS (MUGWORT)
ALLERGENE PRINCIPAL RECOMBINE DU POLLEN D'i(ARTEMISIA VULGARIS) (ARMOISE)
Publication Language: German
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 3464
Publication Year: 1999

15/6/43 (Item 12 from file: 349)
00508857
ALTERATION OF AMINO ACID COMPOSITIONS IN SEEDS
MODIFICATION DE COMPOSITIONS D'ACIDES AMINES DANS DES GRAINES
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 7355
Publication Year: 1999

15/6/44 (Item 13 from file: 349)
00488770 **Image available**
INCREASING THE DIGESTIBILITY OF FOOD PROTEINS BY THIOREDOXIN REDUCTION
AMELIORATION DE LA DIGESTIBILITE DES PROTEINES ALIMENTAIRES PAR REDUCTION PAR LA THIOREDOXINE
Publication Language: English
Fulltext Availability:
Detailed Description

Claims
Fulltext Word Count: 38007
Publication Year: 1999

15/6/45 (Item 14 from file: 349)
00476374
COMPOSITIONS THAT SPECIFICALLY BIND TO COLORECTAL CANCER CELLS AND METHODS OF USING THE SAME
COMPOSITIONS QUI SE LIENT SPECIFIQUEMENT AUX CELLULES CANCEREUSES COLORECTALES ET UTILISATION DE CES COMPOSITIONS
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 36129
Publication Year: 1999

15/6/46 (Item 15 from file: 349)
00445243
COMPOSITIONS THAT BIND TO PANCREATIC CANCER CELLS AND METHODS OF USING THE SAME
COMPOSITIONS QUI SE FIXENT SUR LES CELLULES CANCEREUSES PANCREATIQUES ET LEUR MODE D'UTILISATION
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 23391
Publication Year: 1998

15/6/47 (Item 16 from file: 349)
00416399
PEPTIDE WITH INHIBITORY ACTIVITY TOWARDS PLANT PATHOGENIC FUNGI
PEPTIDE POSSEDANT UNE ACTION INHIBITRICE A L'ENCONTRE DE CHAMPIGNONS PATHOGENES DE PLANTES
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 20033
Publication Year: 1998

15/6/48 (Item 17 from file: 349)
00394280 **Image available**
ALTERATION OF AMINO ACID COMPOSITIONS IN SEEDS
MODIFICATION DE COMPOSITIONS D'ACIDES AMINES DANS DES GRAINES
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 15668
Publication Year: 1997

15/6/49 (Item 18 from file: 349)
00359094
PYRULARIA THIONIN CONTAINING IMMUNOTOXINS AND IMMUNOTOXIN-LIKE CONJUGATES
THIONINE PYRULARIA CONTENANT DES IMMUNOTOXINES AINSI QUE DES CONJUGUES SIMILAIRES AUX IMMUNOTOXINES
Publication Language: English
Fulltext Availability:
Detailed Description
Claims

Fulltext Word Count: 12214
Publication Year: 1996

15/6/50 (Item 19 from file: 349)
00330288
NEUTRALIZATION OF FOOF ALLERGENS BY THIOREDOXIN
NEUTRALISATION D'ALLERGENES ALIMENTAIRES PAR LA THIOREDOXINE
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 32222
Publication Year: 1996

15/6/51 (Item 20 from file: 349)
00326460
COMPOSITIONS AND METHODS FOR THE ABROGATION OF CELLULAR PROLIFERATION UTILIZING THE HUMAN IMMUNODEFICIENCY VIRUS Vpr PROTEIN
COMPOSITIONS ET PROCEDES PERMETTANT D'INTERROMPRE UNE PROLIFERATION CELLULAIRE A L'AIDE DE LA PROTEINE Vpr DU VIH
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 19863
Publication Year: 1996

15/6/52 (Item 21 from file: 349)
00300078
ANTIMICROBIAL PROTEINS
PROTEINES ANTIMICROBIENNES
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 7917
Publication Year: 1995

15/6/53 (Item 22 from file: 349)
00293545
COMPOSITIONS THAT SPECIFICALLY BIND TO COLORECTAL CANCER CELLS AND METHODS OF USING THE SAME
COMPOSITIONS SE FIXANT SPECIFIQUEMENT A DES CELLULES CANCEREUSES COLO-RECTALES ET PROCEDES D'UTILISATION
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 42481
Publication Year: 1995

15/6/54 (Item 23 from file: 349)
00286605
ANTIMICROBIAL PROTEINS
PROTEINES ANTIMICROBIENNES
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 11516
Publication Year: 1995

15/6/55 (Item 24 from file: 349)
00286604
COMPOSITIONS OF FUSION PROTEINS CONTAINING METALLOTHIONEIN AND TARGETING-PROTEIN STRUCTURAL COMPONENTS
COMPOSITIONS DE PROTEINES DE FUSION CONTENANT DES COMPOSANTS STRUCTURAUX DE METALLOTHIONEINE ET DE PROTEINE DE CIBLAGE
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 9406
Publication Year: 1995

15/6/56 (Item 25 from file: 349)
00267908 **Image available**
HIGH LYSINE DERIVATIVES OF ALPHA-HORDOTHIONIN
DERIVES D'ALPHA-HORDOTHIONINE A HAUTE TENEUR EN LYSINE
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 5778
Publication Year: 1994

15/6/57 (Item 26 from file: 349)
00234016
USE OF THIOL REDOX PROTEINS FOR REDUCING DISULFIDE BONDS
UTILISATION DE PROTEINES D'OXYDOREDUCTION A BASE DE THIOL POUR REDUIRE DES LIAISONS BISULFURES
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 33674
Publication Year: 1993

15/6/58 (Item 27 from file: 349)
00230900
BIOCIDAL PROTEINS
PROTEINES BIOCIDES
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 13654
Publication Year: 1993

15/6/59 (Item 28 from file: 349)
00230335
BIOCIDAL PROTEINS
PROTEINES BIOCIDES
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 9050
Publication Year: 1993

15/6/60 (Item 29 from file: 349)
00224457
BIOCIDAL PROTEINS
PROTEINES BIOCIDES
Publication Language: English

Fulltext Availability:
  Detailed Description
  Claims
Fulltext Word Count: 7952
Publication Year: 1992

15/6/61 (Item 30 from file: 349)
00218464
BIOCIDAL PROTEINS
PROTEINES BIOCIDES
Publication Language: English
Fulltext Availability:
  Detailed Description
  Claims
Fulltext Word Count: 6953
Publication Year: 1992

15/6/62 (Item 31 from file: 349)
00208988
PROTEINACEOUS ANTI-DENTAL PLAQUE AGENTS
AGENTS D'ELIMINATION DE LA PLAQUE DENTAIRE A BASE DE PROTEINES
Publication Language: English
Fulltext Availability:
  Detailed Description
  Claims
Fulltext Word Count: 13547
Publication Year: 1992

15/6/63 (Item 32 from file: 349)
00159749
USE OF THIOREDOXIN, THIOREDOXIN-DERIVED, OR THIOREDOXIN-LIKE DITHIOL PEPTIDES IN HAIR CARE PREPARATION
UTILISATION DE PEPTIDES DE DITHIOL DE THIOREDOXINE, DERIVES OU ANALOGUES DE THIOREDOXINE, DANS DES PREPARATIONS DE SOIN DES CHEVEUX
Publication Language: English
Fulltext Availability:
  Detailed Description
  Claims
Fulltext Word Count: 6278
Publication Year: 1989

15/6/64 (Item 1 from file: 5)
0006236844 BIOSIS NO.: 198886076765
TYROSINE HYDROGEN-BONDING AND ENVIRONMENTAL EFFECTS IN PROTEINS PROBED BY UV RESONANCE RAMAN SPECTROSCOPY
1988

15/6/65 (Item 1 from file: 348)
00642457
HIGH LYSINE DERIVATIVES OF ALPHA-HORDOTHIONIN
DERIVATE VON ALPHA-HORDOTHIONIN MIT HOHEREM BEHALT AN LYSIN
DERIVES D'ALPHA-HORDOTHIONINE A HAUTE TENEUR EN LYSINE
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	200137	515
CLAIMS B	(German)	200137	492
CLAIMS B	(French)	200137	578
SPEC B	(English)	200137	4391
Total word count - document A			0
Total word count - document B			5976
Total word count - documents A + B			5976

15/6/66 (Item 2 from file: 348)
00579685
BIOCIDAL PROTEINS
BIOZIDE PROTEINE
PROTEINES BIOCIDES
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	200133	408
CLAIMS B	(German)	200133	344
CLAIMS B	(French)	200133	431
SPEC B	(English)	200133	5803
Total word count - document A			0
Total word count - document B			6986
Total word count - documents A + B			6986

15/6/67 (Item 3 from file: 348)
00537586
Natural and synthetic proteins with inhibitory activity towards pathogenic microorganisms.
Naturliche und synthetische Proteine mit inhibitorischer Aktivitat gegen pathogene Mikroorganismen.
Proteines naturelles et synthetiques avec activite inhibitrice contre des microorganismes pathogenes.
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	959
SPEC A	(English)	EPABF1	5135
Total word count - document A			6094
Total word count - document B			0
Total word count - documents A + B			6094

15/6/68 (Item 4 from file: 348)
00245328
Therapeutic and related uses of dithiol peptides.
Therapeutische und verwandte Verwendungen von Dithiol-Peptiden.
Utilisations therapeutiques et apparentees des dithiol peptides.
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	285
SPEC A	(English)	EPABF1	3897
Total word count - document A			4182
Total word count - document B			0
Total word count - documents A + B			4182

15/6/69 (Item 5 from file: 348)
00224186
Protein-folding enzyme.
Protein faltendes Enzym.
Enzyme de pliage de proteines.
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	452
SPEC A	(English)	EPABF1	5235
Total word count - document A			5687
Total word count - document B			0
Total word count - documents A + B			5687

15/6/70 (Item 6 from file: 348)
00220520
Folding disulfide-cross-linkable proteins.
In Falten gelegte Proteine, durch Disulfid gebunden.
Proteines pliantes liees par pont disulfure en croix.
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	515
SPEC A	(English)	EPABF1	4071
Total word count - document A			4586
Total word count - document B			0
Total word count - documents A + B			4586

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$0.00 1 Type(s) in Format 6
$0.00 1 Types
$0.48 Estimated cost File155
$6.20 1.050 DialUnits File654
$6.30 9 Type(s) in Format 3
$7.50 30 Type(s) in Format 6
$13.80 39 Types
$20.00 Estimated cost File654
$3.61 0.287 DialUnits File399
$3.61 Estimated cost File399
$1.96 0.413 DialUnits File349
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$8.00 32 Types
$9.96 Estimated cost File349
$4.99 0.293 DialUnits File340
$4.99 Estimated cost File340
$0.86 0.150 DialUnits File5
$0.00 1 Type(s) in Format 6
$0.00 1 Types
$0.86 Estimated cost File5
$1.55 0.341 DialUnits File348
$3.40 2 Type(s) in Format 3
$1.50 6 Type(s) in Format 6
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$6.45 Estimated cost File348
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$0.45 0.120 DialUnits File65
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$0.07 0.030 DialUnits File203
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$0.24 0.045 DialUnits File156
$0.24 Estimated cost File156
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$2.81 0.090 DialUnits File398
$2.81 Estimated cost File398
$0.63 0.030 DialUnits File357
$0.63 Estimated cost File357
$1.34 0.126 DialUnits File73
$1.34 Estimated cost File73
$0.32 0.072 DialUnits File50
$0.32 Estimated cost File50
OneSearch, 18 files, 3.597 DialUnits FileOS
$0.80 TELNET
$58.32 Estimated cost this search
$58.32 Estimated total session cost 3.597 DialUnits

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### Status: Signed Off. (3 minutes)

### Status: Path 1 of [Dialog Information Services via Modem]

### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID pto-dialog)
Trying 31060000009998...Open

DIALOG INFORMATION SERVICES
PLEASE LOGON:
 ******** HHHHHHHH SSSSSSSS?
### Status: Signing onto Dialog
 ********
ENTER PASSWORD:
 ******** HHHHHHHH SSSSSSSS? ********
Welcome to DIALOG
### Status: Connected


Dialog level 04.20.00D

Reconnected in file OS 26feb05 10:16:16
* * *

SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R) 1951-2005/Feb W3
         (c) format only 2005 The Dialog Corp.
*File 155: Medline has been reloaded; acession numbers have changed.
Please see HELP NEWS 154.
  File 654:US Pat.Full. 1976-2005/Feb 24
         (c) Format only 2005 The Dialog Corp.
  File 399:CA SEARCH(R) 1967-2005/UD=14209
         (c) 2005 American Chemical Society
*File 399: Use is subject to the terms of your user/customer agreement.
Alert feature enhanced for multiple files, etc. See HELP ALERT.
  File 349:PCT FULLTEXT 1979-2002/UB=20050217,UT=20050210
         (c) 2005 WIPO/Univentio
  File 340:CLAIMS(R)/US Patent 1950-05/Feb 24
         (c) 2005 IFI/CLAIMS(R)
*File 340: 2004 Reload is online as of October 6, 2004.  Pricing
changes effective October 1, 2004.  See HELP NEWS 340 for details.
  File   5:Biosis Previews(R) 1969-2005/Feb W3
         (c) 2005 BIOSIS
*File   5: Price change effective Jan 1, 2005. Enter HELP
RATES 5 for details.
  File 348:EUROPEAN PATENTS 1978-2005/Feb W03
         (c) 2005 European Patent Office
  File 347:JAPIO Nov 1976-2004/Oct(Updated 050208)
         (c) 2005 JPO & JAPIO
*File 347: JAPIO data problems with year 2000 records are now fixed.
Alerts have been run.  See HELP NEWS 347 for details.
  File  65:Inside Conferences 1993-2005/Feb W3
         (c) 2005 BLDSC all rts. reserv.
  File  35:Dissertation Abs Online 1861-2005/Feb
         (c) 2005 ProQuest Info&Learning
  File 342:Derwent Patents Citation Indx 1978-05/200510
         (c) 2005 Thomson Derwent
  File 203:AGRIS 1974-2004/Nov
         Dist by NAL, Intl Copr. All rights reserved
  File 156:ToxFile 1965-2005/Feb W3
         (c) format only 2005 The Dialog Corporation
*File 156: Updating of ToxFile has resumed, with
UD=20041205.
  File  94:JICST-EPlus 1985-2005/Jan W2
         (c)2005 Japan Science and Tech Corp(JST)
```

File 398:Chemsearch 1957-2005/Jan
(c) 2005 Amer.Chem.Soc.
*File 398: Use is subject to the terms of your user/customer agreement.
Problems with SORT. RANK charge added. See HELP RATES 398.
File 357:Derwent Biotech Res. _1982-2005/Feb W4
(c) 2005 Thomson Derwent & ISI
File 73:EMBASE 1974-2005/Feb W3
(c) 2005 Elsevier Science B.V.
*File 73: Price change effective Jan 1, 2005. Enter HELP
RATES 73 for details.
File 50:CAB Abstracts 1972-2005/Jan
(c) 2005 CAB International

Set	Items	Description

Cost is in DialUnits
?ds

Set	Items	Description
S1	373	ALPHA (2N) THIONIN?
S2	313	RD (unique items)
S3	279736	'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION'
S4	53755	'EDTA'
S5	44607	R1-R4
S6	79	S2 AND (S3 OR S4 OR S5 OR EDTA? OR ETHYLENEDIAMINE?)
S7	51	S6/2002:2005
S8	28	S6 NOT S7
S9	689	E3-E33
S10	1	'PUROTHIORINS'
S11	689	S9 OR S10
S12	128	S11 AND (EDTA? OR ETHYLENE? OR ETHYLENEDIAMINE? OR EDTA)
S13	50	S12/2002:2005
S14	78	S12 NOT S13
S15	70	S14 NOT S8

?t s15/9/1

15/9/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08856644 PMID: 2610351
Histochemical localization of cysteine-rich proteins by tissue printing on nitrocellulose.
Pont-Lezica R F; Varner J E
Department of Biology, Washington University, St. Louis, Missouri 63130.
Analytical biochemistry (UNITED STATES) Nov 1 1989, 182 (2) p334-7,
ISSN 0003-2697 Journal Code: 0370535
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
A rapid technique for the histochemical localization of cysteine-rich proteins in plant tissues was developed. It is based on the immediate transfer of proteins to nitrocellulose membranes when a fresh cut organ is pressed against the membrane surface. The print was labeled for cysteine-rich proteins by reduction and alkylation of cysteinyl residues with dansylated iodoacetamide [N-iodoacetyl-N'-(-5-sulfo-1-naphthyl) **ethylenediamine**]. The S-carboxymethylated proteins were visualized by their fluorescence when excited with 360 nm light.
Tags: Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.
Descriptors: *Collodion; *Cysteine--analysis--AN; *Histocytochemistry --methods--MT; *Proteins--analysis--AN; Hordeum--analysis--AN; Lectins --analysis--AN; Membranes, Artificial; Naphthalenesulfonates; Oxidation-Reduction; Plant Lectins; Plant Proteins--analysis--AN; Potatoes

--analysis--AN
CAS Registry No.: 0 (Lectins); 0 (Naphthalenesulfonates); 0 (Plant Lectins); 0 (Plant Proteins); 0 (Proteins); 36930-63-9 (1,5-I-AEDANS); 52-90-4 (Cysteine); 9004-70-0 (Collodion); 9009-72-7 (purothionin)
Record Date Created: 19900222
Record Date Completed: 19900222
?t s15/3,kwic/2-70
>>>KWIC option is not available in file(s): 398, 399

15/3,KWIC/2 (Item 1 from file: 654)
DIALOG(R)File 654:US Pat.Full.

0004925643
Derwent Accession: 2002-010726
Compositions and methods for identifying and targeting cancer cells of alimentary canal origin
Inventor: Scott Waldman, INV
Jason Park, INV
Stephanie Schulz, INV
Correspondence Address: Mark DeLuca, Esq. WOODCOCK WASHBURN KURTZ, MACKIEWICZ & NORRIS LLP One Liberty Place - 46th Floor, Philadelphia, PA, 19103, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20010036635	A1	20011101	US 2001819247	20010327
Provisional				US 60-192229	20000327

Fulltext Word Count: 30220

Summary of the Invention:
...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein- specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well as...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens...

Non-exemplary or Dependent Claim(s):
...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/3 (Item 2 from file: 654)
DIALOG(R)File 654:US Pat.Full.

0004918035
Derwent Accession: 2002-381264
Compositions and methods for identifying and targeting cancer cells of alimentary canal origin
Inventor: Scott Waldman, INV
Jason Park, INV
Stephanie Schulz, INV
Correspondence Address: Mark DeLuca, Esq. WOODCOCK WASHBURN KURTZ, MACKIEWICZ & NORRIS LLP One Liberty Place - 46th Floor, Philadelphia, PA, 19103, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20010029019	A1	20011011	US 2001819249	20010327
Provisional				US 60-192229	20000327

Fulltext Word Count: 30022

Summary of the Invention:
...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well as...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens...

Non-exemplary or Dependent Claim(s):
...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/4 (Item 3 from file: 654)
DIALOG(R)File 654:US Pat.Full.

0004903757 **IMAGE Available
Derwent Accession: 1993-100978
Biocidal proteins
Inventor: Willem Broekaert, INV
Bruno Cammue, INV

CERTIFICATE OF CORRECTION
C/ **Methods of identifying and detecting pancreatic cancer**
; IN VITRO DIAGNOSIS OF TUMORS IN HUMANS; ANALYZING PREFERENTIAL ORGAN TISSUE FOR PRESENCE OF CHOLECYSTOKININ MESSENGER RIBONUCLEIC ACIDS, PRESENCE OF CHOLECYSTOKININ MESSENGER RIBONUCLEIC ACIDS INDICATES HUMAN HAS A TUMOR
Inventor: Weinberg, David, Philadelphia, PA
Waldman, Scott A., Ardmore, PA
Barber, Michael T., Paoli, PA
Biswas, Sanjoy, Philadelphia, PA
Assignee: Thomas Jefferson University(02), Philadelphia, PA
Jefferson, Thomas University (Code: 06943)
Examiner: Eyler, Yvonne (Art Unit: 162)
Assistant Examiner: Holleran, Anne L.
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6187536	A	20010213	US 9825534	19980218
Provisional				US 60-38063	19970218

Fulltext Word Count: 23168

Summary of the Invention:
...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well as ...

15/3,KWIC/8 (Item 7 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4427576
Derwent Accession: 2000-687452
Utility
C/ **X-ray guided drug delivery**
; TARGETING A TISSUE; EXPOSURE OF TISSUE TO IONIZING RADIATION
Inventor: Hallahan, Dennis E., Nashville, TN
Assignee: Vanderbilt University(02), Nashville, TN
Vanderbilt University (Code: 88418)
Examiner: Schwartzman, Robert A. (Art Unit: 166)
Assistant Examiner: Sandals, William
Law Firm: Jenkins & Wilson, P.A.

	Publication Number	Kind	Date	Application Number	Filing Date

Main Patent	US 6159443	A	20001212	US 99302456	19990429

Fulltext Word Count: 26146

Summary of the Invention:
...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin...

Description of the Invention:
...by staining for factor VIII. Confluent cells were harvested with 0.1% collagenase 0.01% **EDTA** and subcultured at a ratio of 1:3. HUVECs were used at third passage;

Non-exemplary or Dependent Claim(s):
...4 fluorouracil, melphalan, chlorambucil, a nitrogen mustard, cyclophosphamide, cis-platinum, vindesine, vinca alkaloids, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin, doxorubicin, daunomycin, vinblastine...daunorubicin, cytosine arabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cyclophosphamide, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin, doxorubicin, daunomycin, vinblastine...

15/3,KWIC/9 (Item 8 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4384858
Derwent Accession: 1999-180474
Utility
C/ **Compositions that specifically bind to colorectal cancer cells and methods of using the same**
; IN VITRO METHOD OF DIAGNOSING METASTASIZED COLORECTAL CANCER BY DETECTING GENE EXPRESSION OF COLORECTAL CANCER-ASSOCIATED TRANSCRIPT-1, AN ALTERNATE FORM OF HEAT-STABLE TOXIN RECEPTOR, IN CELLS OF SAMPLE
Inventor: Waldman, Scott A., Ardmore, PA
Pearlman, Joshua M., Philadelphia, PA
Barber, Michael T., Paoli, PA
Schulz, Stephanie, West Chester, PA
Parkinson, Scott J., Philadelphia, PA
Assignee: Thomas Jefferson University(02), Philadelphia, PA
Jefferson, Thomas University (Code: 06943)
Examiner: Eyler, Yvonne (Art Unit: 162)
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6120995	A	20000919	US 97908643	19970807

Fulltext Word Count: 34307

Description of the Invention:
...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses,

2-D14 comprises **purothionin** conjugated to SEQ ID NO:2...hours at room temperature in 0.4M Tris-HCl, pH 8.0 and 1 mM **EDTA** . Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and... activity of the ST peptide. [sup]111 In is rapidly and potently chelated by either **EDTA** (**ethylenediaminetetraacetic** acid) or DTPA (**diethylenetriaminepetaacetic** acid). DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The [sup]111 In-DTPA is ...

Non-exemplary or Dependent Claim(s):
...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platin, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platin, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platin, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/16 (Item 15 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4085182
Derwent Accession: 1997-480228
Utility
C/ **Alteration of amino acid compositions in seeds**
Inventor: Jung, Rudolf, Des Moines, IA
Hastings, Craig, Perry, IA
Coughlan, Sean, Des Moines, IA
Hu, David, Johnston, IA
Assignee: Pioneer Hi-Bred International, Inc.(02), Des Moines, IA
Pioneer Hi-Bred International Inc (Code: 17947)
Examiner: LeGuyader, John (Art Unit: 165)
Assistant Examiner: McGarry, Sean
Law Firm: Pioneer Hi-Bred International, Inc.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5850016	A	19981215	US 96618911	19960320

Fulltext Word Count: 12377
Description of the Invention:
...invention include plant proteins enriched in cysteine but not methionine, such as the wheat endosperm **purothionine** (Mak and Jones; Can. J. Biochem.; Vol. 22; p. 83J; (1976); incorporated herein in its... pH 5.2 and concentrated in the dialysis bags to about 100 ml with dry **polyethyleneglycol** (PEG 8000). Precipitated contaminating globulin proteins are removed by centrifugation at 6000Xg for 15 min...

15/3,KWIC/17 (Item 16 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4057665

TES 5 for details.
  File 34:SciSearch(R) Cited Ref Sci 1990-2005/Feb W3
        (c) 2005 Inst for Sci Info
*File 34: Price change effective Jan 1, 2005. Enter HELP
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  File 35:Dissertation Abs Online 1861-2005/Jan
        (c) 2005 ProQuest Info&Learning
  File 48:SPORTDiscus 1962-2005/May
        (c) 2005 Sport Information Resource Centre
  File 65:Inside Conferences 1993-2005/Feb W3
        (c) 2005 BLDSC all rts. reserv.
  File 71:ELSEVIER BIOBASE 1994-2005/Feb W2
        (c) 2005 Elsevier Science B.V.
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        (c) 2005 Elsevier Science B.V.
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  File 91:MANTIS(TM) 1880-2005/Feb
        2001 (c) Action Potential
  File 94:JICST-EPlus 1985-2005/Jan W2
        (c)2005 Japan Science and Tech Corp(JST)
  File 98:General Sci Abs/Full-Text 1984-2004/Dec
        (c) 2005 The HW Wilson Co.
  File 135:NewsRx Weekly Reports 1995-2005/Feb W3
        (c) 2005 NewsRx
*File 135: New newsletters are now added. See Help News135 for the
complete list of newsletters.
  File 144:Pascal 1973-2005/Feb W2
        (c) 2005 INIST/CNRS
*File 144: Price change effective Jan 1, 2005. Enter HELP
RATES 144 for details.
  File 149:TGG Health&Wellness DB(SM) 1976-2005/Feb W2
        (c) 2005 The Gale Group
  File 156:ToxFile 1965-2005/Feb W3
        (c) format only 2005 The Dialog Corporation
*File 156: Updating of ToxFile has resumed, with
UD=20041205.
  File 159:Cancerlit 1975-2002/Oct
        (c) format only 2002 Dialog Corporation
*File 159: Cancerlit is no longer updating.
Please see HELP NEWS159.
  File 162:Global Health 1983-2005/Jan
        (c) 2005 CAB International
  File 164:Allied & Complementary Medicine 1984-2005/Feb
        (c) 2005 BLHCIS
  File 172:EMBASE Alert 2005/Feb W2
        (c) 2005 Elsevier Science B.V.
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  File 266:FEDRIP 2004/Nov
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  File 369:New Scientist 1994-2005/Feb W2
        (c) 2005 Reed Business Information Ltd.
  File 370:Science 1996-1999/Jul W3
        (c) 1999 AAAS
*File 370: This file is closed (no updates). Use File 47 for more current
information.
  File 399:CA SEARCH(R) 1967-2005/UD=14209
        (c) 2005 American Chemical Society
*File 399: Use is subject to the terms of your user/customer agreement.
Alert feature enhanced for multiple files, etc. See HELP ALERT.
  File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
        (c) 1998 Inst for Sci Info
*File 434: Price change effective Jan 1, 2005. Enter HELP
RATES 434 for details.
  File 444:New England Journal of Med. 1985-2005/Feb W3
        (c) 2005 Mass. Med. Soc.

File 467:ExtraMED(tm) 2000/Dec
(c) 2001 Informania Ltd.
*File 467: F467 no longer updates; see Help News467. 7.

Set	Items	Description

Cost is in DialUnits
?ds

Set	Items	Description
S1	369	'THIONIN'
S2	21702	'EDTA'
S3	2176	'ETHYLENEDIAMINES' OR 'ETHYLENEDIAMINETETRAACETIC ACID'
S4	2173	R1-R2
S5	2	S1 AND (S2 OR S3 OR S4)
S6	211502	EDTA? OR ETHYLENEDIAMINE?
S7	7121	THIONIN?
S8	77	S6 AND S7
S9	56	RD (unique items)
S10	5	S9/2000:2005
S11	51	S9 NOT S10

?t s11/9/1 2 3 4 5 8 7 10 11 13 14 22 51

11/9/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12120265 PMID: 9421187
Mechanisms by which thionin **induces susceptibility of S49 cell membranes to extracellular phospholipase A2.**
Wilson H A; Huang W; Waldrip J B; Judd A M; Vernon L P; Bell J D
Department of Zoology, Brigham Young University, Provo, UT 84602, USA.
Biochimica et biophysica acta (NETHERLANDS) Nov 15 1997, 1349 (2) p142-56, ISSN 0006-3002 Journal Code: 0217513
Contract/Grant No.: GM-49710; GM; NIGMS
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Whereas cells normally resist attack by PLA2, they become susceptible under certain pathological conditions. To ascertain the regulatory mechanisms that induce cellular susceptibility to PLA2, the effect of **thionin** on S49 cells was examined in the presence of PLA2. **Thionin** alone was unable to evoke hydrolysis of the lipid bilayer. Likewise, the addition of PLA2 alone caused production of only a minimal amount of free fatty acid. However, **thionin** and PLA2 together resulted in significant hydrolysis of the cell membrane. **Thionin** caused perturbation of the bilayer structure as suggested by the changes in the emission spectra of laurdan and the permeability of the membrane to propidium iodide. These changes correlated quantitatively with the susceptibility of the lipid bilayer to PLA2. Furthermore, **thionin** induced a modest increase in intracellular Ca2+. The source of this Ca2+ was the extracellular fluid since **EDTA** in the extracellular medium inhibited the Ca2+ influx. Moreover, cobalt chloride, a universal Ca2+ channel blocker, prevented the rise in intracellular Ca2+, the uptake of propidium iodide, and the susceptibility to PLA2 induced by **thionin** . In contrast, the changes in the laurdan emission caused by the **thionin** were not affected by the cobalt. Furthermore, incubation of the cells with the calcium ionophore A23187 also caused the cells to become susceptible to PLA2. We hypothesize that **thionin** causes S49 cell membranes to become susceptible to PLA2 by a Ca2+-dependent perturbation of the bilayer structure.
Tags: Research Support, U.S. Gov't, P.H.S.
Descriptors: *Phenothiazines--pharmacology--PD; *Phospholipases A --pharmacology--PD; Animals; Arachidonic Acid--metabolism--ME; Calcimycin --pharmacology--PD; Calcium--metabolism--ME; Cell Membrane--metabolism--ME;

Lipid Bilayers--metabolism--ME; Lymphoma--metabolism--ME; Mice; Tumor Cells, Cultured
CAS Registry No.: 0 (Lipid Bilayers); 0 (Phenothiazines); 506-32-1 (Arachidonic Acid); 52665-69-7 (Calcimycin); 581-64-6 (thionine); 7440-70-2 (Calcium)
Enzyme No.: EC 3.1.1.- (Phospholipases A)
Record Date Created: 19980115
Record Date Completed: 19980115

11/9/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10106631 PMID: 7680580
Thionin **staining of paraffin and plastic embedded sections of cartilage.**
Bulstra S K; Drukker J; Kuijer R; Buurman W A; van der Linden A J
Department of Orthopaedic Surgery, University Hospital Maastricht, State University of Limburg, The Netherlands.
Biotechnic & histochemistry - official publication of the Biological Stain Commission (UNITED STATES) Jan 1993, 68 (1) p20-8, ISSN 1052-0295 Journal Code: 9107378
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The usefulness of **thionin** for staining cartilage sections embedded in glycol methacrylate (GMA) and the effect of decalcification on cartilage sections embedded in paraffin and GMA were assessed. Short decalcification periods using 5% formic acid or 10% **EDTA** did not influence the staining properties or the morphology of cartilage matrix and chondrocytes. The standard stain safranin O-fast green for differential staining of cartilage was used as control in these experiments. Prolonged exposure of safranin O stained sections to fast green resulted in disappearance of the safranin O stained matrix, thereby hampering the quantitative measurement of negatively charged glycosaminoglycans (GAG). **Thionin** stained evenly throughout all cartilage layers, independent of the staining times. In contrast to safranin O, **thionin** did not show metachromasia in nondehydrated cartilage sections, which made it more suitable for assessing cartilage quality in GMA embedded cartilage. To evaluate the selectivity of **thionin** staining in cartilage, chondroitinase ABC and trypsin digestions were carried out. **Thionin** staining was prevented by these enzymes in the territorial matrix, representing the interlacunar network and the chondrocyte capsule. Staining with **thionin** of the interterritorial matrix was only slightly reduced, possibly representing keratan sulfate and hyaluronic acid in cartilage of elderly patients. Comparison of **thionin** stained GMA embedded cartilage with safranin O stained paraffin embedded sections showed significant similarity in optical densitometry, indicative of the specificity of **thionin** bound to negatively charged GAG in cartilage. In GMA embedded cartilage morphology was relatively intact compared to paraffin embedded sections due to less shrinkage of chondrocytes and the interlacunar network.
Tags: Comparative Study
Descriptors: *Cartilage, Articular--cytology--CY; *Methacrylates; *Paraffin Embedding; *Phenothiazines--metabolism--ME; Aged; Cartilage, Articular--anatomy and histology--AH; Chondroitin Lyases--metabolism--ME; Decalcification Technique; Glycosaminoglycans--metabolism--ME; Humans; Phenazines; Plastic Embedding; Sensitivity and Specificity; Staining and Labeling--methods--MT
CAS Registry No.: 0 (Glycosaminoglycans); 0 (Methacrylates); 0 (Phenazines); 0 (Phenothiazines); 477-73-6 (safranine T); 581-64-6 (thionine); 868-77-9 (hydroxyethyl methacrylate)
Enzyme No.: EC 4.2.2.- (Chondroitin Lyases)
Record Date Created: 19930412

Record Date Completed: 19930412

11/9/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05131256 PMID: 594487
[Selective photooxidation of methionine, tyrosine and tryptophane using thionine and toluidine blue as sensitizers (author's transl)]
Fotooxidacion selectiva de metionina, tirosina y triptofano utilizando tionina y azul de toluidina como fotosensibilizadores.
Iborra J L; Llorca F I; Pastor R F; Garcia J V
Revista espanola de fisiologia (SPAIN) Dec 1977, 33 (4) p297-304, ISSN 0034-9402 Journal Code: 0404475
Publishing Model Print
Document type: Journal Article ; English Abstract
Languages: SPANISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Thionine and toluidine blue were used as sentizers on photooxidation processes of methionine, tryosine and tryptophane. They were more effective than methylene blue. Methionine was photooxidized to sulfoxide and tryptophane to kinurenine. A tyrosine-sensitizer addition compound was postulated. Dye concentration, pH, temperature and **EDTA** presence conditions were determined on each one of the modification reactions. Methionine at acid pH was selectively modified. On the basis of obtained results and published references, a direct interaction of singlet oxygen with methionine and tryptophase and the excited dye with tyrosine was respectively discussed.
Tags: Comparative Study
Descriptors: *Amino Acids--metabolism--ME; *Photosynthesis--drug effects --DE; *Tyrosine--metabolism--ME; Ergothioneine--pharmacology--PD; Methionine--metabolism--ME; Oxidation-Reduction--drug effects--DE; Photic Stimulation; Tolonium Chloride--pharmacology--PD; Tryptophan--metabolism --ME
CAS Registry No.: 0 (Amino Acids); 497-30-3 (Ergothioneine); 55520-40-6 (Tyrosine); 63-68-3 (Methionine); 73-22-3 (Tryptophan); 92-31-9 (Tolonium Chloride)
Record Date Created: 19780223
Record Date Completed: 19780223

11/9/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04488011 PMID: 806084
Mechanism of photoreduction of thiazine dyes by EDTA studied by flash photolysis III. Consequences of a newly found pKT of thionine on the mechanism in basic solutions.
Bonneau R; Pereyre J
Photochemistry and photobiology (ENGLAND) Mar 1975, 21 (3) p173-7, ISSN 0031-8655 Journal Code: 0376425
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Descriptors: *Dyes; *Edetic Acid; *Light; *Oxidation-Reduction; *Phenothiazines; *Photolysis; *Thiazines--metabolism--ME; Imines
CAS Registry No.: 0 (Dyes); 0 (Imines); 0 (Phenothiazines); 0 (Thiazines); 60-00-4 (Edetic Acid)
Record Date Created: 19750829
Record Date Completed: 19750829

11/9/5 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0003236407 BIOSIS NO.: 198171055366
CHARACTERIZATION OF THE RED LIGHT INDUCED REDUCTION OF A PARTICLE ASSOCIATED B TYPE CYTOCHROME FROM CORN IN THE PRESENCE OF METHYLENE BLUE
AUTHOR: WIDELL S (Reprint); BRITZ S J; BRIGGS W R
AUTHOR ADDRESS: DEP PLANT PHYSIOL, BOX 7007, S-22007, LUND 7, SWEDEN** SWEDEN
JOURNAL: Photochemistry and Photobiology 32 (5): p669-678 1980
ISSN: 0031-8655
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Methylene blue transfers electrons to a membrane-associated b-type cytochrome in particulate fractions from corn coleoptiles. The Km for methylene blue is less than 1 .mu.M under optimal conditions. This reaction is destroyed by boiling, but not by 7 M urea. Kinetic analyses of the influence of light intensity on cytochrome reduction suggest that a 1st order photochemical reaction is limiting. Free **EDTA** may serve as an electron donor in this system at least at high methylene blue and protein concentrations. The photoactivity does not coincide either with mitochondrial or endoplasmic reticulum markers, and may be localized in plasma membrane. There is an estimated 5 .times. 10-10 mol photoreducible cytochrome/g coleoptile tissue. Studies on the effect of pH on the reaction in the presence of methylene blue or **thionine** indicate that dye photoreduction itself is not rate-limiting. Wavelength dependence studies suggest that it is methylene blue monomer and not dimer which mediates the reaction. Although O2 is apparently required for the reaction, neither superoxide nor excited singlet oxygen appear to be involved. The reaction mechanism is still unknown.

REGISTRY NUMBERS: 61-73-4: METHYLENE BLUE; 60-00-4: **EDTA** ; 7782-44-7: OXYGEN
DESCRIPTORS: COLEOPTILES ELECTRON TRANSFER **EDTA** WAVELENGTH DEPENDENCE OXYGEN REQUIREMENT
DESCRIPTORS:
MAJOR CONCEPTS: Bioenergetics--Biochemistry and Molecular Biophysics; Enzymology--Biochemistry and Molecular Biophysics; Radiation Biology
BIOSYSTEMATIC NAMES: Gramineae--Monocotyledones, Angiospermae, Spermatophyta, Plantae
COMMON TAXONOMIC TERMS: Angiosperms; Monocots; Plants; Spermatophytes; Vascular Plants
CHEMICALS & BIOCHEMICALS: METHYLENE BLUE; **EDTA** ; OXYGEN
CONCEPT CODES:
10012 Biochemistry - Gases
10060 Biochemistry studies - General
10064 Biochemistry studies - Proteins, peptides and amino acids
10065 Biochemistry studies - Porphyrins and bile pigments
10510 Biophysics - Bioenergetics: electron transport and oxidative phosphorylation
10604 External effects - Light and darkness
10802 Enzymes - General and comparative studies: coenzymes
51516 Plant physiology - Light and radiation effects
52504 Agronomy - Grain crops
BIOSYSTEMATIC CODES:
25305 Gramineae

11/9/8 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

01233933 Genuine Article#: GG799 Number of References: 91
Title: MODEL PHOTOGALVANIC SYSTEMS FOR THE CONVERSION OF SOLAR-ENERGY
Author(s): SHAGISULTANOVA GA; TAITS AM; TIMONOV AM
Corporate Source: AI GERTSEN STATE TEACHERS INST/LENINGRAD//USSR/
Journal: JOURNAL OF APPLIED CHEMISTRY OF THE USSR, 1990, V63, N12, P 2427-2440
Language: ENGLISH Document Type: ARTICLE
Geographic Location: UNION OF SOVIET SOCIALIST REPUBLICS
Subfile: SciSearch; CC PHYS--Current Contents, Physical, Chemical & Earth Sciences
Journal Subject Category: CHEMISTRY, APPLIED
Identifiers--KeyWords Plus: **THIONINE** -COATED ELECTRODE; **ETHYLENEDIAMINETETRAACETIC** ACID SYSTEM; PHOTO-CHEMICAL MECHANISMS; PHOTOELECTROCHEMICAL CELL; METHYLENE-BLUE; RATE CONSTANT; IRON SYSTEM; POWER; EFFICIENCY; KINETICS
Research Fronts: 89-6804 002 (PHOTOGALVANIC CELL FOR SOLAR-ENERGY CONVERSION; PHENOSAFRANIN DYE; METHYLENE BLUE-NITRILOTRIACETIC ACID SYSTEM)
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11/9/7 (Item 2 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02513289 Genuine Article#: LG689 Number of References: 0
(NO REFS KEYED)

Title: USE OF TRITON X-100 IN A PHOTOGALVANIC CELL FOR SOLAR-ENERGY CONVERSION AND STORAGE - THIONINE EDTA **SYSTEM**

Author(s): AMETA SC; CHITTORA AK; KHAMESRA S; AMETA R
Corporate Source: SUKHADIA UNIV,UNIV COLL SCI,DEPT CHEM,15 RADHEYSHYAM ST,BHRAMPOLE GATE/UDAIPUR 313001//INDIA/

Journal: ARABIAN JOURNAL FOR SCIENCE AND ENGINEERING, 1992, V17, N4A (OCT), P477-480
ISSN: 0377-9211
Language: ENGLISH Document Type: ARTICLE
Geographic Location: INDIA
Subfile: SciSearch; CC ENGI--Current Contents, Engineering, Technology & Applied Sciences
Journal Subject Category: MULTIDISCIPLINARY SCIENCES

Abstract: **Thionine , ethylenediaminetetraacetic** acid, and Triton X-100 (Tx) have been used as photosensitizer, reductant, and surfactant, respectively in a photogalvanic cell for solar energy conversion. The

photocurrent and photopotential generated by this cell were 70.0 muA and 888.0 mV, respectively. The effect of various parameters on the electrical output of the cell was studied, and current-voltage characteristics of the cell have also been observed.

11/9/10 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

08896137 PASCAL No.: 90-0064116
Use of the thionine **- EDTA system in photogalvanic cells for solar energy conversion**
AMETA S C; SADHNA KHAMESRA; SUSHILA LODHA; RAMESHWER AMETA
Sukhadia univ., dep. chemistry, Udaipur 313 001, India
Journal: Journal of photochemistry and photobiology. A. Chemistry, 1989 , 48 (1) 81-86
ISSN: 531731 CODEN: JPPCEJ Availability: CNRS-15990A
No. of Refs.: 19 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: Switzerland
Language: English
Description d'une cellule solaire utilisant de la **thionine** comme photosensibilisant et l' **EDTA** comme reducteur: caracteristiques potentiometriques en fonction de la concentration en **thionine** , en **EDTA** , du pH et de la geometrie de la couche et mecanismes de reaction

English Descriptors: Solar cell; Energy conversion; Electrical characteristic; Photosensitizer; Medium effect; Potentiometry; Chemical concentration; Experimental study; Reaction mechanism; **EDTA**
French Descriptors: Cellule solaire; Conversion energie; Caracteristique electrique; Photosensibilisant; Effet milieu; Potentiometrie; Concentration chimique; Etude experimentale; Mecanisme reaction; **EDTA** ; **Thionine**

Classification Codes: 230C02B; 001D06C02

11/9/11 (Item 2 from file: 144)
DIALOG(R)File 144:Pascal

05746232 PASCAL No.: 84-0247280
Electrochemistry and photoelectrochemistry of dye-incorporated clay-modified electrode
KAMAT P V
Univ. Notre Dame, radiation lab., Notre Dame IN 46556, USA
Journal: Journal of electroanalytical chemistry and interfacial electrochemistry, 1984, 163 (1-2) 389-394
ISSN: 0022-0728 Availability: CNRS-1150
No. of Refs.: 13 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: Switzerland
Language: English
Etudes dans le cas de **thionine** incorporee dans des couches minces d'argile fixees sur des electrodes de SnO SUB 2 et de Pt. Resultats obtenus par etudes de voltammetrie cyclique, ainsi que donnees sur la generation de l'effet photoelectrochimique sous l'effet d'un rayonnement de lambda >460 nm et en milieu aqueux contenant de l' **EDTA** 0,2 M

English Descriptors: Photoelectrochemistry; Semiconductor materials; Electrodes; Transition metal; Metal Oxides; Platinum; Thin layer electrode; Modified material; Chemical uptake; Thiazine dye; Electrochemical reaction; Cyclic voltammetry; Photoelectrochemical reaction; Aqueous solution; Organic solvent; Tin IV Oxides; Clay

French Descriptors: Photoelectrochimie; Semiconducteur; Electrode; Metal transition; Metal Oxyde; Platine-ACT; Electrode couche mince; Materiau modifie; Fixation chimique; Colorant thiazinique; Reaction electrochimique; Voltammetrie cyclique; Reaction photoelectrochimique; Solution aqueuse; Solvant organique; Etain IV Oxyde-ACT; Argile; **Thionine** ; Violet Lauth; **EDTA** -SUB

Classification Codes: 001C01H06

11/9/13 (Item 4 from file: 144)
DIALOG(R)File 144:Pascal

04862032 PASCAL No.: 83-0108558
Effect of DNA and other polyanions on the EDTA **induced photoreduction of** thionine
MEDINI KANTA PAL; MANNA P C
Univ. Kalyani, dep. chemistry, Kalyani 741235, India
Journal: Makromol. Chem., 1982, 183 (11) 2811-2821
ISSN: 0025-116X Availability: CNRS-4111
No. of Refs.: 12 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: Switzerland
Language: English
Influence de l'ADN, de l'ARN et du polystyrenesulfonate

English Descriptors: DNA; Polyanion; Reduction; Photochemical reaction; Styrenesulfonate polymer; RNA; Basic dye

French Descriptors: DNA; Polyanion; Reduction; Reaction photochimique; Styrenesulfonate polymere; RNA; Colorant basique; **Thionine** -ENT; Tetracetique acide(**ethylenediamine**); Utilisation

Classification Codes: 780A02E

11/9/14 (Item 5 from file: 144)
DIALOG(R)File 144:Pascal

04003372 PASCAL No.: 75-0028838
MECHANISM OF PHOTOREDUCTION OF THIAZINE DYES BY EDTA **STUDIED BY FLASH PHOTOLYSIS. III. CONSEQUENCES OF A NEWLY FOUND PK SUB T OF** THIONINE **ON THE MECHANISM IN BASIC SOLUTIONS**
BONNEAU R; PEREYRE J
LAB. CHIM. PHYS. A, UNIV. BORDEAUX I, 33405 TALENCE,FRANCE
Journal: PHOTOCHEM. AND PHOTOBIOL., 1975, 21 (3) 173-177
Availability: CNRS-9410
No. of Refs.: 13 REF.
Document Type: P (SERIAL) ; A (ANALYTIC)
Country of Publication: UNITED KINGDOM
Language: ENGLISH
EQUILIBRE ENTRE LES FORMES NEUTRE ET MONOCATIONIQUE DE LA **THIONINE** TRIPLET, SUP 3 T ET SUP 3 TH SUP + (PK SUB T =8,9). PAS D'EQUILIBRE IDENTIQUE AVEC LE BLEU DE METHYLENE. DIFFERENCE DUE A LEUR PHOTOREDUCTION DIFFERENTE PAR **EDTA** . LE RENDEMENT QUANTIQUE DE FORMATION DU COLORANT SEMI REDUIT EST PROPORTIONNEL AU RENDEMENT DE REDUCTION GLOBAL. MECANISMES

English Descriptors: METHYLENE BLUE; ORGANIC DYE; MONOCYCLIC COMPOUND; ACIDITY CONSTANT; **EDTA** ; SULFUR NITROGEN HETEROCYCLE; REACTION MECHANISM ; FLASH PHOTOLYSIS; PHOTOCHEMICAL REACTION; REDUCTION; QUANTUM YIELD
English Generic Descriptors: PHYSICAL CHEMISTRY; PHOTOCHEMISTRY

French Descriptors: COLORANT ORGANIQUE; THIAZINE; BLEU METHYLENE; **THIONINE** ; HETEROCYCLE SOUFRE AZOTE; COMPOSE MONOCYCLIQUE; **EDTA** ; REDUCTION;

REACTION PHOTOCHIMIQUE; CONSTANTE ACIDITE; RENDEMENT QUANTIQUE; PHOTOLYSE ECLAIR; MECANISME REACTION
French Generic Descriptors: CHIMIE PHYSIQUE; PHOTOCHIMIE

Classification Codes: 170A14B02A

11/9/22 (Item 13 from file: 144)
DIALOG(R)File 144:Pascal

00112095 PASCAL No.: 73-0032420
INDICADORES FOTO-REDOX EN AMPEROMETRA. DETERMINACIONES INDIRECTAS DE ANIONES CON ETILENODIAMINO TETRAACETO DIHYDROGENO DISODICO
(INDICATEURS PHOTO-REDOX EN AMPEROMETRIE. DOSAGE INDIRECT DES ANIONS AVEC L' EDTA -H SUB 2 NA SUB 2)
SIERRA F; SANCHEZ-PEDRENO C; PEREZ RUIZ T; MARTINEZ LOZANO C
C.S.I.C., DEP. QUIM. ANAL.,DE MURCIA
Journal: INFORM. QUIM. ANAL., 1973, 27 (2) 93-98
Availability: CNRS-2711
No. of Refs.: 6 REF.
Document Type: P (SERIAL)
Country of Publication: SPAIN
Language: SPANISH Summary Language: ENGLISH
EMPLOI DE **THIONINE** COMME INDICATEUR REDOX-PHOTOCHIMIQUE POUR LE TITRAGE INDIRECT DE CRO SUB 4 SUP 2- , SO SUB 4 SUP 2- , PO SUB 4 H SUB 2 SUP - ET (FE(CN) SUB 6) SUP 4-

English Descriptors: AMPEROMETRY; CHEMICAL ANALYSIS;CHEMICAL COMPOSITION; CHROMATES; ANIONIC COMPLEX; **EDTA** ; ANALYTICAL INDICATOR; REDOX INDICATOR ; PHOSPHATES; ABSORPTION SPECTROMETRY;ABSORPTION SPECTROSCOPY; SULFATES
English Generic Descriptors: ANALYTICAL CHEMISTRY
French Descriptors: **THIONINE** ; INDICATEUR ANALYTIQUE; CHROMATE; SULFATE; PHOSPHATE; FER II COMPOSE; FER II COMILEXE; CONPLEXE CYANO; COMPLEXE ANIONIQUE; ANALYSE CHIMIQUE; AMPEROMETRIE; TITRAGE AMPEROMETRIQUE; **EDTA** ; INDICATEUR REDOX; SPECTROMETRIE ABSORPTION; ANALYSE PAR; METHODE INDIRECTE; CYANO; ANALYSE
French Generic Descriptors: CHIMIE ANALYTIQUE

Classification Codes: 170C05B06

11/9/51 (Item 3 from file: 434)
DIALOG(R)File 434:SciSearch(R) Cited Ref Sci

01388440 Genuine Article#: CW889 Number of References: 20
Title: DETERMINATION OF IODIDE TRACES USING INHIBITORY EFFECT IN PHOTOCHEMICAL INTERACTION OF THIONINE **AND** ETHYLENEDIAMINETETRAACETIC **ACID**
Author(s): SIERRA F; SANCHEZPEDRENO C; PEREZRUIZ T; MARTINEZLOZANO C; HERNANDEZCORDOBA M
Corporate Source: FAC CIENCIAS MURCIA,CSIC,CTR COORDINADO,DEPT QUIM ANAL/MURCIA//SPAIN/
Journal: ANALES DE QUIMICA, 1977, V73, N1, P67-70
Language: SPANISH Document Type: ARTICLE
Geographic Location: SPAIN
Subfile: SciSearch; CC PHYS--Current Contents, Physical, Chemical & Earth Sciences
Journal Subject Category: CHEMISTRY
Cited References:
BARKLEY RA, 1960, V32, P154, ANALYT CHEMISTRY
BOGNAR J, 1963, V37, P381, ACTA CHIM ACAD SCI H
CHANEY AL, 1950, V22, P939, ANALYT CHEMISTRY
CHANEY AL, 1940, V12, P179, IND ENG CHEM ANALYT

DUBRAVCIC M, 1955, V80, P146, ANALYST
ESPENSON JH, 1964, V86, P5101, J AM CHEM SOC
FAURE J, 1966, V63, P621, J CHIM PHYS
JOUSSOTDUBIEN J, 1963, V91, P1214, J CHIM PHYS
KAZUO S, 1969, P119, TOHUKU U SERV
LEIN A, 1951, V23, P1507, ANALYT CHEMISTRY
MASSAE N, 1969, V42, P2464, B CHEM SOC JAPAN
OSTER G, 1957, V79, P4836, J AM CHEM SOC
SANDELL EB, 1934, V56, P1426, J AM CHEM SOC
SIERRA F, 1972, V68, P1091, AN QUIMICA
SIERRA F, 1974, V70, P595, AN QUIMICA
SIERRA F, 1975, V78, P498, ANAL CHIM ACTA
SIERRA F, 1975, V78, P277, ANALYT CHIM
SIERRA F, 1971, V25, P73, INFORM QUIM ANAL
SIERRA F, 1973, V27, P93, INFORM QUIM ANAL
SIERRA F, TO BE PUBLISHED

?t s11/3,kwic/25-48
>>>KWIC option is not available in file(s): 399

11/3,KWIC/25 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

121234606 CA: 121(20)234606w JOURNAL
Use of cetylpyridinium chloride in photogalvanic cell for solar energy conversion and storage: thionine-EDTA system
AUTHOR(S): Ameta, Suresh C.; Lodha, Anita; Sahasi, Sapna; Ameta, Rameshwar
LOCATION: Univ. Coll. Sci., Sukhadia Univ., Udaipur, 313 001, India
JOURNAL: Proc. Natl. Acad. Sci., India, Sect. A DATE: 1994 VOLUME: 64 NUMBER: 1 PAGES: 43-8 CODEN: PAIAA3 ISSN: 0369-8203 LANGUAGE: English

11/3,KWIC/26 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

118224926 CA: 118(23)224926z JOURNAL
(1,2-Bis(2-hydroxyphenyl)ethylenediamine)dichloroplatinum(II), a new compound for the therapy of ovarian cancer. III. Detailed evaluation of the antitumor activity of the enantiomeric complexes on the human NIH:OVCAR-3 ovarian cancer cell line
AUTHOR(S): Bernhardt, Gunther; Gust, Ronald; Reile, Herta; Vom Orde, Hans Dieter; Mueller, Richard; Keller, Christoph; Spruss, Thilo; Schoenenberger, Helmut; Burgemeister, Thomas; et al.
LOCATION: Inst. Pharm., Univ. Regensburg, Regensburg, Germany, W-8400
JOURNAL: J. Cancer Res. Clin. Oncol. DATE: 1992 VOLUME: 118 NUMBER: 3 PAGES: 209-15 CODEN: JCROD7 ISSN: 0171-5216 LANGUAGE: English

11/3,KWIC/27 (Item 3 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

117082983 CA: 117(9)82983u JOURNAL
Dichloro-(1-(hydroxyphenyl)-2-phenylethylenediamine)platinum(II) complexes: testing on the human ovarian cancer cell lines NIH: OVCAR 3 and SK OV 3
AUTHOR(S): Bernhardt, Guenther; Mueller, Richard; Gust, Ronald; Reile, Herta; Keller, Christoph; Spruss, Thilo; Schoenenberger, Helmut
LOCATION: Inst. Pharm., Univ. Regensburg, Regensburg, Germany, W-8400
JOURNAL: Arch. Pharm. (Weinheim, Ger.) DATE: 1992 VOLUME: 325 NUMBER: 2 PAGES: 93-9 CODEN: ARPMAS ISSN: 0365-6233 LANGUAGE: English

11/3,KWIC/28 (Item 4 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

113086948 CA: 113(10)86948b JOURNAL
Photogalvanic effect in systems containing basic dyes
AUTHOR(S): Pezza, Leonardo; Neumann, Miguel Guilermo; Gessner, Fergus
LOCATION: Inst. Biocienc., UNESP, 15100, Sao Jose do Rio Preto, Brazil
JOURNAL: Ecletica Quim. DATE: 1989 VOLUME: 14, PAGES: 27-37 CODEN: ECQUDX ISSN: 0100-4670 LANGUAGE: Portuguese

11/3,KWIC/29 (Item 5 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

107069877 CA: 107(8)69877m JOURNAL
The reaction between thionine blue and EDTA as a photochemical pretreatment in determinations of chromium(VI) and peroxydisulfate
AUTHOR(S): Martinez Lozano, C.; Tomas Martinez, V.; Yague, E.
LOCATION: Cent. Coord., CSIC, Spain,
JOURNAL: Afinidad DATE: 1987 VOLUME: 44 NUMBER: 408 PAGES: 115-18
CODEN: AFINAE ISSN: 0001-9704 LANGUAGE: Spanish

11/3,KWIC/30 (Item 6 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

106226561 CA: 106(26)226561c JOURNAL
Thionine blue as a photoredox indicator in titrations with EDTA. Determinations of metal ions, mixtures, and anions
AUTHOR(S): Perez Ruiz, T.; Martinez Lozano, C.; Tomas, V.; Yague, E.
LOCATION: Fac. Cienc. Quim. Mat., Univ. Murcia, Murcia, Spain,
JOURNAL: Quim. Anal. (Barcelona) DATE: 1986 VOLUME: 5 NUMBER: 2
PAGES: 164-79 CODEN: QUANEL ISSN: 0212-0569 LANGUAGE: Spanish

11/3,KWIC/31 (Item 7 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

101113892 CA: 101(14)113892q JOURNAL
Thionine and ferric chelate compounds as coupled mediators in microbial fuel cells
AUTHOR(S): Tanaka, Kazuko; Vega, Carmen A.; Tamamushi, Reita
LOCATION: Inorg. Chem. Lab., Inst. Phys. Chem. Res., Wako, Japan, 351
JOURNAL: Bioelectrochem. Bioenerg. DATE: 1983 VOLUME: 11 NUMBER: 4-6
PAGES: 289-97 CODEN: BEBEBP ISSN: 0302-4598 LANGUAGE: English

11/3,KWIC/32 (Item 8 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

99178976 CA: 99(22)178976e JOURNAL
Photochemical energy conversion by a thiazine photosynthetic-photoelectrochemical cell
AUTHOR(S): Pan, R. L.; Bhardwaj, R.; Gross, E. L.
LOCATION: Dep. Biochem., Ohio State Univ., Columbus, OH, 43210, USA
JOURNAL: J. Chem. Technol. Biotechnol., Chem. Technol. DATE: 1983
VOLUME: 33A NUMBER: 1 PAGES: 39-48 CODEN: JCTTDW ISSN: 0142-0356
LANGUAGE: English

11/3,KWIC/33 (Item 9 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

98071284 CA: 98(9)71284e JOURNAL
Effect of DNA and other polyanions on the EDTA induced photoreduction of thionine
AUTHOR(S): Pal, Medini Kanta; Manna, Pravash C.
LOCATION: Fac. Sci., Univ. Kalyani, Kalyani, 741235, India
JOURNAL: Makromol. Chem. DATE: 1982 VOLUME: 183 NUMBER: 11 PAGES: 2811-21 CODEN: MACEAK ISSN: 0025-116X LANGUAGE: English

11/3,KWIC/34 (Item 10 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

93060423 CA: 93(6)60423v JOURNAL
The use of thionine as a photoredox indicator in amperometric titrations of the mercury(I) ion with polyaminopolycarboxylated agents
AUTHOR(S): Sierra, F.; Cebrian, A.; Hidalgo de Cisneros, J. L. H.
LOCATION: Dep. Quim. Anal., Fac. Cienc. Murcia, Murcia, Spain,
JOURNAL: Afinidad DATE: 1979 VOLUME: 36 NUMBER: 364 PAGES: 515-17
CODEN: AFINAE ISSN: 0001-9704 LANGUAGE: Spanish

11/3,KWIC/35 (Item 11 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

88145569 CA: 88(20)145569u JOURNAL
Determination of periodate with photoreduced thionine
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Perez Ruiz, T.; Martinez Lozano, C.
LOCATION: Dep. Anal. Chem., Univ. Murcia, Murcia, Spain
JOURNAL: Anal. Chim. Acta DATE: 1977 VOLUME: 94 NUMBER: 1 PAGES: 129-33 CODEN: ACACAM ISSN: 0003-2670 LANGUAGE: English

11/3,KWIC/36 (Item 12 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

88083095 CA: 88(12)83095a JOURNAL
Determination of trace iodide by using its inhibiting effect on the photochemical reaction between thionine and EDTA
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Perez Ruiz, T.; Martinez Lozano, C.; Hernandez Cordoba, M.
LOCATION: Dep. Quim. Anal., CSIC, Murcia, Spain
JOURNAL: An. Quim. DATE: 1977 VOLUME: 73 NUMBER: 1 PAGES: 67-70
CODEN: ANQUBU LANGUAGE: Spanish

11/3,KWIC/37 (Item 13 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

86162741 CA: 86(22)162741v JOURNAL
Photoinduced potentials by photoredox systems and the effects of the addition of EDTA
AUTHOR(S): Kaneko, Masao; Yamada, Akira
LOCATION: Inst. Phys. Chem. Res., Wako, Japan
JOURNAL: Rikagaku Kenkyusho Hokoku DATE: 1976 VOLUME: 52 NUMBER: 6 PAGES: 210-15 CODEN: RKKHAO LANGUAGE: Japanese

11/3,KWIC/38 (Item 14 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

85195209 CA: 85(26)195209k TECHNICAL REPORT

Evaluation of some thionine redox systems as potential regenerative photogalvanic batteries
AUTHOR(S): Fine, Dwight A.; Fletcher, Aaron N.
LOCATION: Nav. Weapons Cent., China Lake, Calif.
JOURNAL: U. S. NTIS, AD Rep. DATE: 1976 NUMBER: AD-A021424 PAGES: 25 pp. CODEN: XADRCH LANGUAGE: English CITATION: Gov. Rep. Announce. Index (U. S.) 1976, 76(9), 121 AVAIL: NTIS

11/3,KWIC/39 (Item 15 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

82154984 CA: 82(23)154984d JOURNAL
Photochemical determination of a new acid-base equilibrium of thionine in its triplet state. Application to the photoreactivity of thiazine dyes in aqueous solution
AUTHOR(S): Bonneau, Roland; Pereyre, Josette; Joussot-Dubien, Jacques
LOCATION: Lab. Chim. Phys. A, Univ. Bordeaux I 351, Talance, Fr.
JOURNAL: Mol. Photochem. DATE: 1974 VOLUME: 6 NUMBER: 3 PAGES: 245-52
CODEN: MLPCBL LANGUAGE: English

11/3,KWIC/40 (Item 16 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

81019300 CA: 81(4)19300s PATENT
Photochromic polymers
INVENTOR(AUTHOR): Hasegawa, Haruo
ASSIGNEE: Ricoh Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho JP 7401690 DATE: 740109
APPLICATION: Japan JP 7238911 DATE: 720418
PAGES: 5 pp. CODEN: JKXXAF CLASS: 26(3)F115

11/3,KWIC/41 (Item 17 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

79100138 CA: 79(16)100138a JOURNAL
Photochemical redox indicators in amperometry. Indirect determinations of anions with disodium dihydrogen ethylenediaminetetraacetate
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Perez Ruiz, T.; Martinez Lozano, C.
LOCATION: Cons. Super. Invest. Cient., Murcia, Spain
JOURNAL: Inform. Quim. Anal. DATE: 1973 VOLUME: 27 NUMBER: 2 PAGES: 93-8 CODEN: IFQAAZ LANGUAGE: Spanish

11/3,KWIC/42 (Item 18 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

79043647 CA: 79(8)43647q JOURNAL
Mechanism of photoreduction of thiazine dyes by EDTA studied by flash photolysis. I
AUTHOR(S): Bonneau, Roland; Joussot-Dubien, Jacques; Faure, Jean
LOCATION: Lab. Chim. Phys. A, Univ. Bordeaux I, Talence, Fr.
JOURNAL: Photochem. Photobiol. DATE: 1973 VOLUME: 17 NUMBER: 5
PAGES: 313-19 CODEN: PHCBAP LANGUAGE: English

11/3,KWIC/43 (Item 19 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

78066493 CA: 78(10)66493e JOURNAL
Photoreduction indicators in amperometry. Titration of mercury(II), zinc(II), and calcium(II) with ethylenediaminetetraacetic acid
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Martinez Lozano, C.; Perez Ruiz, T.
LOCATION: Dep. Quim. Anal., Fac. Cienc. Murcia, Murcia, Spain
JOURNAL: An. Quim. DATE: 1972 VOLUME: 68 NUMBER: 9-10 PAGES: 1091-6
CODEN: ANQUBU LANGUAGE: Spanish

11/3,KWIC/44 (Item 20 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

76080559 CA: 76(14)80559w JOURNAL
Use of photosensitizers in some volumetric determinations of reduction
AUTHOR(S): Martinez Lozano, Maria Del C.
LOCATION: Univ. Murcia, Murcia, Spain
JOURNAL: An. Univ. Murcia, Cienc. DATE: 1970 VOLUME: 28 NUMBER: 1-2-3-4 PAGES: 127-97 CODEN: AUMCB5 LANGUAGE: Spanish

11/3,KWIC/45 (Item 21 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

74008313 CA: 74(2)8313c JOURNAL
Photochemical reduction of thionine and other thiazine dyes by Co(II)EDTA complex in a heterogeneous system
AUTHOR(S): Singhal, G. S.; Rabinowitch, Eugene
LOCATION: Chem. Dep., State Univ. New York, Albany, N. Y.
JOURNAL: J. Chem. Phys. DATE: 1970 VOLUME: 53 NUMBER: 10 PAGES: 4109-10 CODEN: JCPSA6 LANGUAGE: English

11/3,KWIC/46 (Item 22 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

73051836 CA: 73(10)51836s JOURNAL
Formation and stabilization of high valence states in silver
AUTHOR(S): Sanchez=Manzanares, Jose A.
JOURNAL: An. Univ. Murcia, Cienc. DATE: 1968 VOLUME: 26 NUMBER: 1-4 PAGES: 57-161 CODEN: AUMCB5 LANGUAGE: Spanish

11/3,KWIC/47 (Item 23 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

72122159 CA: 72(24)122159r JOURNAL
Photopolymerization using methylene blue-reducing agent system and its application to photoelectrochemical cell
AUTHOR(S): Kamiya, Nobuyuki; Okawara, Makoto
LOCATION: Res. Lab. Resour. Util., Tokyo Inst. Technol., Tokyo, Japan
JOURNAL: Kogyo Kagaku Zasshi DATE: 1969 VOLUME: 72 NUMBER: 12 PAGES: 2639-44 CODEN: KGKZA7 LANGUAGE: Japanese

11/3,KWIC/48 (Item 24 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

72084849 CA: 72(16)84849f JOURNAL
Photochemical reduction of thionine by cobalt(II) EDTA complex in water-ether emulsion
AUTHOR(S): Srinivasan, V.; Rabinowitch, E.

LOCATION: Dep. of Bot., Univ. of Illinois, Urbana, Ill.
JOURNAL: J. Chem. Phys. DATE: 1970 VOLUME: 52 NUMBER: 3 PAGES: 1165-8
CODEN: JCPSA6 LANGUAGE: English

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$0.11 0.033 DialUnits File155
$0.84 4 Type(s) in Format 9
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$0.95 Estimated cost File155
$0.06 0.011 DialUnits File5
$2.00 1 Type(s) in Format 9
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$2.06 Estimated cost File5
$0.74 0.033 DialUnits File34
$12.86 2 Type(s) in Format 9
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$13.60 Estimated cost File34
$0.05 0.011 DialUnits File35
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$0.04 0.011 DialUnits File94
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$0.03 0.011 DialUnits File98
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$0.06 0.011 DialUnits File135
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$0.09 0.022 DialUnits File144
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$0.06 0.011 DialUnits File156
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$0.03 0.011 DialUnits File159
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$0.05 Estimated cost File162
$0.04 0.011 DialUnits File164
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$66.00 24 Types
$67.40 Estimated cost File399
$0.49 0.022 DialUnits File434
$6.43 1 Type(s) in Format 9
$6.43 1 Types
$6.92 Estimated cost File434
$0.05 0.011 DialUnits File444
$0.05 Estimated cost File444

11/3,KWIC/40 (Item 16 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

81019300 CA: 81(4)19300s PATENT
Photochromic polymers
INVENTOR(AUTHOR): Hasegawa, Haruo
ASSIGNEE: Ricoh Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho JP 7401690 DATE: 740109
APPLICATION: Japan JP 7238911 DATE: 720418
PAGES: 5 pp. CODEN: JKXXAF CLASS: 26(3)F115

11/3,KWIC/41 (Item 17 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

79100138 CA: 79(16)100138a JOURNAL
Photochemical redox indicators in amperometry. Indirect determinations of anions with disodium dihydrogen ethylenediaminetetraacetate
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Perez Ruiz, T.; Martinez Lozano, C.
LOCATION: Cons. Super. Invest. Cient., Murcia, Spain
JOURNAL: Inform. Quim. Anal. DATE: 1973 VOLUME: 27 NUMBER: 2 PAGES: 93-8 CODEN: IFQAAZ LANGUAGE: Spanish

11/3,KWIC/42 (Item 18 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

79043647 CA: 79(8)43647q JOURNAL
Mechanism of photoreduction of thiazine dyes by EDTA studied by flash photolysis. I
AUTHOR(S): Bonneau, Roland; Joussot-Dubien, Jacques; Faure, Jean
LOCATION: Lab. Chim. Phys. A, Univ. Bordeaux I, Talence, Fr.
JOURNAL: Photochem. Photobiol. DATE: 1973 VOLUME: 17 NUMBER: 5
PAGES: 313-19 CODEN: PHCBAP LANGUAGE: English

11/3,KWIC/43 (Item 19 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

78066493 CA: 78(10)66493e JOURNAL
Photoreduction indicators in amperometry. Titration of mercury(II), zinc(II), and calcium(II) with ethylenediaminetetraacetic acid
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Martinez Lozano, C.; Perez Ruiz, T.
LOCATION: Dep. Quim. Anal., Fac. Cienc. Murcia, Murcia, Spain
JOURNAL: An. Quim. DATE: 1972 VOLUME: 68 NUMBER: 9-10 PAGES: 1091-6
CODEN: ANQUBU LANGUAGE: Spanish

11/3,KWIC/44 (Item 20 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

76080559 CA: 76(14)80559w JOURNAL
Use of photosensitizers in some volumetric determinations of reduction
AUTHOR(S): Martinez Lozano, Maria Del C.
LOCATION: Univ. Murcia, Murcia, Spain
JOURNAL: An. Univ. Murcia, Cienc. DATE: 1970 VOLUME: 28 NUMBER: 1-2-3-4 PAGES: 127-97 CODEN: AUMCB5 LANGUAGE: Spanish

11/3,KWIC/45 (Item 21 from file: 399)

Detailed Description
... capable of binding antigen or antibodies. well-known supports or carriers, include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modif ied cellulose, polyacrylamide, agarose, and magnetite.

The nature of...metals can be attached to the TNF specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediaminetetraacetic** acid (**EDTA**).

The antibody also can be detectably labeled by coupling to a chemiluminescent compound. The presence...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...plus protease inhibitors: aprotinin, leupeptin, pepstatin A, each at 2 gg/ml; PMSF, 1mM, and **EDTA** , 1mM). Cell suspension was incubated on ice for 10 minutes with frequent vortexing, and centrifuged...then centrifuged at 10,000 G. These supernatants were supplemented - 42 with protease inhibitors (PMSF, **EDTA** , EGTA, aprotinin, pepstatin A, and Leupeptin), dialyzed against PBS, then filtered sterilized and kept on...12% glycerol, 12mM HEPES (pH 7.9), 4mM Tris (pH 7.9) , 60mM KCI, 1mM **EDTA** , and 1mM DTT. The oligonucleotides sequences used were obtained from D. Ghosh.

The ...non-denaturing polyacrylamide gel (270 Al 1M Tris, pH 7.9; 80 til O.SM **EDTA** , pH 7.9; 13.2 Al 1M sodium acetate, pH 7.9; 5.33 ml...

...7. 9; 13. 2 ml 1M sodium Acetate, pH 7. 9; 8 ml 0. 5M **EDTA** , pH 8. 0; up to a final volume of 4 liters with ddH20). Gels were...

15/3,KWIC/52 (Item 21 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00300078
ANTIMICROBIAL PROTEINS
PROTEINES ANTIMICROBIENNES
Patent Applicant/Assignee:
ZENECA LIMITED,
BROEKAERT Willem Frans,
CAMMUE Bruno Philippe Angelo,
OSBORN Rupert William,
REES Sarah Bronwen,
Inventor(s):
BROEKAERT Willem Frans,
CAMMUE Bruno Philippe Angelo,
OSBORN Rupert William,
REES Sarah Bronwen,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9518229 A1 19950706
Application: WO 94GB2766 19941219 (PCT/WO GB9402766)
Priority Application: GB 9326424 19931224
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AU BB BG BR BY CA CN CZ FI GE HU JP KG KP KR KZ LK LT LV MD MG MN NO NZ
PL RO RU SI SK TJ TT UA US UZ VN KE MW SD SZ AT BE CH DE DK ES FR GB GR
IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

Publication Language: English
Fulltext Word Count: 7917

Fulltext Availability:
Detailed Description

Detailed Description
... 533-539). Such
proteins, including Sia2 from sorghum and
SURTMM SHEET (RULE 26)
g-l- **purothionin** (g-1P) from wheat, are known to
inhibit insect a-amylase and may be toxic...containing 10 mM NaH 2PO 41
15 mm
Na2HPO 41 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and
1 mM PMSF. The homogenate was squeezed through
cheesecloth and clarified...buffer
contained 200 mM Tris-HCl (pH 8.3), it (w/v) SDS, 1
mM **EDTA** , 0.005t bromophenol blue and, unless
otherwise stated, it (w/v) dithioerythritol (DTE).
Two hundred...

15/3,KWIC/53 (Item 22 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00293545
COMPOSITIONS THAT SPECIFICALLY BIND TO COLORECTAL CANCER CELLS AND METHODS OF USING THE SAME
COMPOSITIONS SE FIXANT SPECIFIQUEMENT A DES CELLULES CANCEREUSES COLO-RECTALES ET PROCEDES D'UTILISATION
Patent Applicant/Assignee:
THOMAS JEFFERSON UNIVERSITY,
WALDMAN Scott A,
Inventor(s):
WALDMAN Scott A,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9511694 A1 19950504
Application: WO 94US12232 19941026 (PCT/WO US9412232)
Priority Application: US 93141892 19931026; US 94305056 19940913
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU JP KE KG KP KR
KZ LK LR LT LU LV MD MG MN MW NL NO NZ PL PT RO RU SD SE SI SK TJ TT UA
US US UZ VN KE MW SD SZ AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 42481

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... g.

cyclophosphamide) , cis-platinum, vindesine (and other vinca
alkaloids), mitomycin and bleomycin. other chemotherapeutics
include: **purothionin** (barley flour oligopeptide) , macromomycin.

1,4-benzoquinone derivatives and trenimon.

Toxins are useful as active...methotrexate,
doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4
flucrouracil, melphalan, chlorambucil, cis-platinum, vindesine,
mitomycin, bleomycin, **purothionin** , macromomycin, 114

CM32 Chromatography
The pullulanase inhibitor sample...30 mM Tris
HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6
ml/fraction) showing pullulanase inhibitory activity were pooled,
concentrated by dialysis against...in wheat flour is about 0.01 %
(Johnson, T.C. et al. (1987), "Reduction of **purothionin** by the wheat
seed thioredoxin system and potential function as a secondary thiol
messenger in...

15/3,KWIC/45 (Item 14 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00476374
COMPOSITIONS THAT SPECIFICALLY BIND TO COLORECTAL CANCER CELLS AND METHODS OF USING THE SAME
COMPOSITIONS QUI SE LIENT SPECIFIQUEMENT AUX CELLULES CANCEREUSES COLORECTALES ET UTILISATION DE CES COMPOSITIONS
Patent Applicant/Assignee:
THOMAS JEFFERSON UNIVERSITY,
Inventor(s):
WALDMAN Scott A,
PEARLMAN Joshua M,
BARBER Michael T,
SCHULZ Stephanie,
PARKINSON Scott J,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9907726 A1 19990218
Application: WO 98US16440 19980807 (PCT/WO US9816440)
Priority Application: US 97908643 19970807
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AU CA JP AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Fulltext Word Count: 36129

Fulltext Availability:
Detailed Description

Detailed Description
... any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite.

The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**).

One skilled in the art would readily recognize other fluorescence-emitting metals as well as...g.

cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon.

Toxins are useful as active...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... ofmethotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine,

mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens.

The vaccines of the present...mM sodium deoxycholate, 3.5 mM sodium dodecyl sulfate, 0.5 @tg/mL leupeptin, ImM **EDTA** , 1 gg/mL pepstatin, and 0.2 MM PMSF.

Protein concentrations were determined using the...

15/3,KWIC/46 (Item 15 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00445243
COMPOSITIONS THAT BIND TO PANCREATIC CANCER CELLS AND METHODS OF USING THE SAME
COMPOSITIONS QUI SE FIXENT SUR LES CELLULES CANCEREUSES PANCREATIQUES ET LEUR MODE D'UTILISATION
Patent Applicant/Assignee:
THOMAS JEFFERSON UNIVERSITY,
WEINBERG David,
WALDMAN Scott A,
BARBER Michael T,
BISWAS Sanjoy,
Inventor(s):
WEINBERG David,
WALDMAN Scott A,
BARBER Michael T,
BISWAS Sanjoy,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9835707 A1 19980820
Application: WO 98US3168 19980218 (PCT/WO US9803168)
Priority Application: US 9738063 19970218
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM
GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX
NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH
GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI
FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 23391

Fulltext Availability:
Detailed Description

Detailed Description
... cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics 5 include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon.

Toxins are useful as active...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain,

floor, Oakland, CA 94607, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
PIETRAS Richard J, 3160 Sawtelle Boulevard, #102, Los Angeles, CA 90066, US, US (Residence), VE (Nationality), (Designated only for: US)
MARQUEZ-GARBAN Diana C, 3464 Lisa Place, Sherman Oaks, CA 91403, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
WOOD William J (agent), Gates & Cooper, 6701 Center Drive West, Suite 1050, Los Angeles, CA 90045, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200162288 A1 20010830 (WO 0162288)
Application: WO 2001US5897 20010223 (PCT/WO US0105897)
Priority Application: US 2000185026 20000225
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 28454

Fulltext Availability:
Detailed Description

Detailed Description
... myeloma lines such as NS-1 or P3NS-1, in the presence of, e.g., **polyethylene** glycol.

The hybridomas or lymphoblastoid cells which secrete antibody of interest can be identified by...doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, n-dtomycin, bleomycin, **purothionin** , macromomycin, 1,4benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, 5 Clostridium...

15/3,KWIC/36 (Item 5 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00784061
NUCLEIC ACID SEQUENCES ENCODING CELL WALL-DEGRADING ENZYMES AND USE TO ENGINEER RESISTANCE TO FUSARIUM AND OTHER PATHOGENS
SEQUENCES D'ACIDE NUCLEIQUE CODANT POUR DES ENZYMES DEGRADANT LES PAROIS CELLULAIRES ET LEUR UTILISATION POUR CREER UNE RESISTANCE AU FUSARIUM ET A D'AUTRES PATHOGENES
Patent Applicant/Assignee:
THE UNITED STATES OF AMERICA as represented by THE SECRETARY OF AGRICULTURE, 1400 Independence Avenue SW, Washington, DC 20250-0302, US , US (Residence), US (Nationality)
NOVO NORDISK BIOTECH INC, 1445 Drew Avenue, Davis, CA 95616-4880, US, US (Residence), US (Nationality)
Inventor(s):
OKUBARA Patricia A, 550 - 34th Street, Richmond, CA 94805, US,
BLECHL Ann E, 1005 Buchanan Street, Albany, CA 94710, US,
HOHN Thomas M, 102 Blakely Drive, Chapel Hill, NC 27514, US,
BERKA Randy M, 3609 Modoc Place, Davis, CA 95616, US,
Legal Representative:
PENDORF Stephan A (et al) (agent), Pendorf & Cutliff, P.O. Box 20445,

Tampa, FL 33622-0445, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200116353 A1 20010308 (WO 0116353)
Application: WO 2000US23802 20000830 (PCT/WO US0023802)
Priority Application: US 99151582 19990830; US 2000224946 20000811; US 2000649747 20000828
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 40894

Fulltext Availability:
Detailed Description
Detailed Description
... leaves were inhibitory to F. solani (Molina et al., 1993). A combination of a wheat **purothionin** and a 2S albumin from radish or oilseed rape was effective against the growth of...in 0.9 M NaCl, 0.09
M Tris-HCl pH 7.6, 6 mM **EDTA** , 0.5% NP-401 IX Denhardt's solution, 1 mM sodium pyrophosphate, 1 mM sodium...were partitioned on 1W agarose in 40 mM TRIS
acetate, pH 8.21 1 mM **EDTA** , stained with an ethidium bromide solution (@1 pg/mL), and visualized by irradiation with an...containing 0.5 M
sucrose, 80 mM potassium chloride, 10 mM TRIS-chloride, 10 mM **EDTA** ,, 4 mM spermine, 1 mM spermidine, pH 9.51 180 mg/L phenylmethylsulfonyl fluoride (added ...ML
of a buffer solution containing 50 mM TRIS-chloride, pH 81 100 mM - 95
EDTA , 100 mM sodium chloride, and 600 @tg of proteinase K. An additional 4 mL of...

...extraction buffer containing 50 mM TRIS-chloride, pH 81 100 mM sodium chloride, 10 mM **EDTA** , pH 81 1OW sodium dodecyl sulfate, and 10 mM beta-mercaptoethanol (added immediately before use...extraction buffer (10 mM sodium chloride, 10 mM TRIS-Cl, pH 9.0j, 1 mM **EDTA**), 2.5 mL phenol (saturated with TRIS buffer at pH 4.31 Fisher #BP1751), and...

...formaldehyde (Fisher #BP531), 20 mM MOPS, pH 7.01 5 mM sodium acetate, 10 mM **EDTA** , and 0.01% bromophenol blue. RNA samples were heated at 600C for 10 to 15...

...was
dissolved in 30 mL of 20 mM MOPS, 5 mM sodium acetate, 10 mM **EDTA** , pH 7.0, to which was added 37 % formaldehyde for a final concentration of 6...

...M sodium phosphate buffer, pH 7.01 0.25 M sodium chloride, and 1 mM **EDTA** , pH 8.0, as recommended by Bio-Rad. Probes for northern blots consisted of partial...consisting of 89 mM TRIS-Cl, pH 8.31 89 mM borate, 1.5 mM
EDTA . The resulting endochitinase cleavage products, shown in FIG. 16, closely matched those expected from the...

15/3,KWIC/37 (Item 6 from file: 349)

activity against *Fusarium* and *Trichoderma* were inactive against *Aspergillus flavus*, *Phytophthora parasitica* and other pathogens (Yun *et al.* (1996). The differential activities of the chitinases are attributed to inherent properties of the enzymes (Sela-Buurlage *et al.* 1993, Brunner *et al.* 1998), to differences in cell wall architecture (Sivan and Chet 1989a, Van Loon 1997) among the fungi, or to other factors.

Other Anti-*Fusarium* Proteins.

Additional types of proteins have been found to have anti-*Fusarium* activity *in vitro* or *in planta*. Boyapati *et al.* (1994) reported a cysteine protease inhibitor from pearl millet that inhibited the growth of *Fusarium moniliforme* in culture. A cysteine-rich polypeptide from *Impatiens balsamina* seeds was active against *F. culmorum* (Tailor *et al.* 1997). Cecropin A, a polypeptide from the *Cecropia* moth, was a potent inhibitor of both *F. moniliforme* and *F. oxysporum* (deLucca *et al.* 1997, Cavallarin *et al.* 1998). Antifungal proteins from seeds of sorghum had activity against *F. moniliforme* (Seetharaman et al. 1997), and two wheat seed proteins of the PR4 family of pathogenesis-related proteins inhibited hyphal growth of *F. culmorum* and *F. graminearum* (Caruso *et al.* 1996). Hu and Reddy (1997) isolated a thaumatin-like protein from *Arabidopsis thaliana* with activity against *F. oxysporum*. Non-specific lipid transfer proteins from barley and maize leaves were inhibitory to *F. solani* (Molina *et al.*, 1993). A combination of a wheat purothionin and a 2S albumin from radish or oilseed rape was effective against the growth of *F. culmorum* in vitro (Terras *et al.* 1993).

Microbial Genes as Anti-Fungal Transgenes in Plants.

A majority of antifungal genes that have been examined both *in vitro* and *in planta* are of plant origin. To our knowledge, there are two examples of genes from fungi that exhibit antifungal activity. Endochitinases from the parasitic fungus *Trichoderma harzianum* conferred activity against *Alternaria alternata* and *B.*

In accordance with this discovery, it is an object of the invention to provide nucleic acid sequences encoding fungal cell wall-degrading enzymes selected from the group consisting of glucanase, exochitinase, and endochitinase; isolated polypeptides having glucanase, endochitinase or exochitinase activity; recombinant nucleic acid molecules including expression vectors encoding polypeptides having cell wall-degrading activity; and cells harboring the recombinant nucleic acid molecules or expression vectors.

It is also an object of the invention to provide transformation vectors comprising a cell wall-degrading recombinant molecule, which vectors are effective for stably introducing the recombinant molecule into a plant.

It is also an object of the invention to provide methods of producing and using polypeptides having glucanase, endochitinase or exochitinase activity.

It is another object of the invention to provide transgenic plants having bacterial or fungal resistance, wherein the resistance is a result of expression of a recombinant nucleic acid molecule of the invention.

A further object of the invention is to provide fungal genes which generate cell wall-degrading enzymes, including proteins having the capability of degrading the glucan and chitin cell wall components of *F. venenatum* and other *Fusarium* species, including *F. graminearum* and *F. culmorum*, the principle causal agents of head blight (scab) in the U.S.

Another object of the invention is expression of the cell wall-degrading enzymes in transgenic monocots, including wheat, barley or oats, to confer partial or complete resistance to *Fusarium* species and/or to other fungal pathogens of wheat and other cereal crops. Such transgenic lines will be useful genetic stocks for generating improved crops.

A still further object of the invention is the provision of novel wheat germplasms that express genes designed to limit the spread of the pathogenic fungus *Fusarium* and indirectly to curtail the accumulation of DON in infected heads.

DEL VAL Gregorio, 6612 Schmidt Lane, No. 4, El Cerrito, CA 94530, US,
LOZANO Rosa M, Olivos de Penanevada, 5-8degreesA, E-28937 Mostoles, ES,
WONG Joshua H, 9 Viewmont Terrace, South San Francisco, CA 94080, US,
YEE Boihon C, 3215 Primrose Lane, Walnut Creek, CA 94598, US,
FRICK Oscar L, 370 Parnassus Avenue, San Francisco, CA 94117, US,
Legal Representative:
SMITH Karen S (et al) (agent), Flehr Hohbach Test Albritton & Herbert LLP, 4 Embarcadero Center, Suite 3400, San Francisco, CA 94111-4187, US
,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200044781 A1 20000803 (WO 0044781)
Application: WO 2000US1958 20000125 (PCT/WO US0001958)
Priority Application: US 99238379 19990127
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 44560

Fulltext Availability:
Detailed Description

Detailed Description
... are soluble cereal seed proteins, rich in cystine. In the Johnson, et al. investigation, wheat **purothionin** was experimentally reduced by NADPH via NADP-thioredoxin reductase (NTR) and thioredoxin h according to Eqs. 2 and 3.

(2) NADPH + Thioredoxin h @-@NADP +
M
Thioredoxin h,ed
(3) **Purothionin** ,,,, + Thioredoxin **Purothionin** ,,
,d
+ Thioredoxin ho,,
Cereal seeds such as wheat, rye, barley, corn, millet, sorghum and rice ...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite
and excising the wound area, there are no known...PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

Other proteins
Purothionin a from bread wheat and **purothionins** ; a-1 and 0 from durum
wheat were kind gifts from Drs. D.D. Kasarda and B.L. Jones, respectively. The **purothionin** a sample contained two members of the **purothionin** family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** a-1 and 0 samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

Routine Method...

...was carried
out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 MM **EDTA** and 16 % glycerol in a final volume of 0. 1 ml. As indicated, 0.7 ...

...extraplastidic proteins, this test has proved useful in several studies.

A case in point is **purothionin** which, when reduced by thioredoxin h activates chloroplast FBPase (Wada, K. et al. (1 98...

...and DSG-2) were found to be effective in enzyme activation; however, they differed from **purothionin** in showing a specificity for NADP-MDH rather than FBPase (Table I).

The a-amylase...2 0
Ovoinhibitor 49 14 1 0
Bovine lung (Aprotinin) 7 3 Trace 2
Thionins
" **Purothionin** -cil 6 4 1 39
** **Purothionin** -0 6 4 Trace 5
tPurothionin-a 6 4 0 14
These values compare to...

...same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** a, 20 ttg or CM-1, 20 ttg was used). The results thus confirmed the...fluorescent band migrating behind thioredoxin.

EXAMPLE 5
Thioredoxin-linked Reduction of Other Trypsin Inhibitors and **Purothionins**
In view of the finding that cystine-rich trypsin inhibitors from seeds can undergo specific...

...a thioredoxin requirement for reduction (data not shown).

In confirmation of earlier results, thioredoxin-reduced **purothionin** consistently activated FBPase and the type tested earlier, **purothionin** -a, failed to activate NADP-MDH (Table I) (Wada, K. et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -a from bread wheat, two **purothionins** previously not examined (**purothionins** a-1 and from durum. wheat) detectably activated NADP-MDH (Table I). The two durum wheat Durothionins also differed in their abilitv to activate FBPase.

The activitv differences between these **purothiorins** were unexpected in view of the strong similarity in their amino acid sequences (Jones, B...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the a-type) by the SDS-PAGE fluorescence procedure.

EXAMPLE 6
Quantitation of Reduction...Procedure
The following concentrations of proteins were used (nmoles): thioredoxin, 0.08; NTR, 0.01; **purothionin** -0, 1.7; DSG-1, 0.7; corn kernel trypsin inhibitor, 1.0; Bowman-Birk...
...difference, other conditions were as in Examples 1

Protein	% Reduction After 20 min	% Reduction After 120 min
Purothionin -0	15	32
DSG-1	22	38
Corn kernel trypsin inhibitor	3	15
Bowman-Birk...		

...CM-1 a-amylase inhibitors (147 and 210%, respectively); corn kernel trypsin inhibitor (424%); and **purothionin** (82, 133, and 120% for the a, al and 0 forms, respectively).

Glutaredoxin was ineffective...

...and ovornucoid
inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two a-arnylase inhibitors (DSG-1,
CM-1), a cystine-rich trypsin inhibitor from plants...0. 1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing
10 mM **EDTA** at 30'C for 2 hours. The concentrations of thioredoxin, NTR, and NADPH were 0and 0.25 mM,
respectively. With DTT as reductant, **EDTA** and components of the NADP/ thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCI, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below.

CM32 Chromatography
The pullulanase inhibitor sample...

...30 mM Tris
HO, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...in wheat flour is about 0.01 % (Johnson, T.C. et al. (1987), "Reduction of **purothionin** by the wheat seed thioredoxin system and potential function as a secondary thiol messenger in...

...mM Tris-HCl pH 7.4 containing 1 liM phenylmethylsulfonyl fluoride (PMSF) and 1 mM **EDTA** -Na] and stirred gently for 30 min at room temperature. The mixture was then centrifuged...

15/3,KWIC/41 (Item 10 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00577322
SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED THEREBY
FRAGMENTS D'ADN DETERMINES SELON LEUR SEQUENCE ET POLYPEPTIDES CORRESPONDANTS CODES PAR LESDITS FRAGMENTS
Patent Applicant/Assignee:
CERES INC, 3007 Malibu Canyon Road, Malibu, CA 90265, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
ALEXANDROV Nickolai, 1404 Oak Trail Street, Thousand Oaks, CA 91320, US, US (Residence), RU (Nationality), (Designated only for: US)
BROVER Vyacheslav, 5916 N. Las Virgenes Road #590, Calabasas, CA 91320, US, US (Residence), RU (Nationality), (Designated only for: US)
CHEN Xianfeng, 12333 Wild Turkey Court, #B, Creve Coeur, MO 63141, US, US (Residence), CN (Nationality), (Designated only for: US)
SUBRAMANIAN Gopalakrishnan, 4205 Peach Slope Road, Moorpark, CA 93021, US , US (Residence), IN (Nationality), (Designated only for: US)
TROUKHAN Maxim E, 1675 Amberwood Drive #2, South Pasadena, CA 91030, US, US (Residence), RU (Nationality), (Designated only for: US)
ZHENG Liansheng, 19212 Circle Gate Drive #201, Germantown, MD 20874, US, US (Residence), CN (Nationality), (Designated only for: US)
Legal Representative:
STEWART Raymond C (et al) (agent), Birch, Stewart, Kolasch & Birch, LLP, P.O. Box 747, Falls Church, VA 22040-0747, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200040695 A2-A3 20000713 (WO 0040695)
Application: WO 2000US466 20000107 (PCT/WO US0000466)
Priority Application: US 99115293 19990108
Designated States:
(Protection type is "patent" unless otherwise stated - for applications

Fulltext Word Count: 11956

Summary of the Invention:
...Biochem, 194:533-539). Such proteins, including SI[alpha]2 from sorghum and [gamma]-1- **purothionin** from wheat, are known to inhibit insect [alpha]-amylase and are toxic to insect larvae...

Description of the Drawings:
...AMP1, the Cb-AMPs, Lc-AFP, Ct-AMP1, sorghum SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed ...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...in 6M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...Sorghum bicolor (Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [gamma]- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in ...

...Lc-AFP, Ct-AMP1, the sorghum [alpha]-amylase inhibitor SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...supplement, respectively. For the purpose of comparison, these tests were performed in parallel with [beta]- **purothionin** , an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...30-fold with both test fungi. In comparison, the IC[sub]50 value of [beta]- **purothionin**

(...AFP2, nor Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/18 (Item 17 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3992551
Derwent Accession: 1996-188141
Utility
C/ **Methods for the identification of compounds capable of inducing the nuclear translocation of a receptor complex comprising the glucocoticoid receptor type II and viral protein R interacting protein ; DETECTING THE HUMAN IMMUNODEFICIENCY VIRUS TYPE I PROTEIN**
Inventor: Weiner, David B., Merion, PA
Refaeli, Yosef, Boston, MA
Assignee: The Trustees of the University of Pennsylvania(02), Philadelphia, PA
Pennsylvania, University of (Code: 64664)
Examiner: Adams, Donald E. (Art Unit: 183)
Assistant Examiner: Parkin, Jeffrey S.
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5763190	A	19980609	US 94309644	19940921

Fulltext Word Count: 15683

Summary of the Invention:

...capable of binding antigen or antibodies. Well-known supports or carriers, include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified cellulose, polyacrylamide, agarose, and magnetite. The nature of the...metals can be attached to the TNF-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediaminetetraacetic** acid (**EDTA**).

...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

Description of the Invention:

...inhibitors: aprotinin, leupeptin, pepstatin A, each at 2 [mu]g/ml; PMSF, 1 mM, and **EDTA** , 1 mM). Cell suspension was incubated on ice for 10 minutes with frequent vortexing, and...were then centrifuged at 10,000 G. These supernatants were supplemented with protease inhibitors (PMSF, **EDTA** , EGTA, aprotinin, pepstatin A, and Leupeptin), dialyzed against PBS, then filtered sterilized and kept on...HEPES (pH 7.9), 4 mM Tris (pH 7.9), 60 mM KCI, 1 mM **EDTA** , and 1 mM DTT. The oligonucleotides sequences used were obtained from D. Ghosh...polyacrylamide gel (270 [mu]l 1M Tris, pH 7.9; 80 [mu]l 0.5M **EDTA** , pH 7.9; 13.2 [mu]l 1M sodium acetate, pH 7.9; 5.33...

...7.9; 13.2 ml 1M sodium Acetate, pH 7.9; 8 ml 0.5M **EDTA** , pH 8.0; up to a final volume of 4 liters with ddH2O). Gels were...

15/3,KWIC/19 (Item 18 from file: 654)

DIALOG(R)File 654:US Pat.Full.

3979152

Derwent Accession: 1995-246394

Utility

C/ **Antimicrobial proteins**

; **PLANT EXTRACTS**

Inventor: Broekaert, Willem Frans, Dilbeek, BE
Cammue, Bruno Philippe Angelo, Alsemberg, BE
Osborn, Rupert William, Twickenham, GB
Rees, Sarah Bronwen, Forest Park, GB

Assignee: Zeneca Agrochemicals(03), GB, England
Zeneca Agrochemicals GB (Code: 45474)

Examiner: Lebuyader, John (Art Unit: 189)

Assistant Examiner: Wang, Andrew

Law Firm: Cushman Darby & Cushman IP Group of Pillsbury Madison & Sutro LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5750504	A	19980512	US 96656318	19960612
PCT	WO 9518229		19950706	WO 94GB2766	19941219
			371:19960612		
			102e:19960612		

Fulltext Word Count: 5652

Summary of the Invention:

...Biochem, 194:533-539). Such proteins, including Si[alpha]2 from sorghum and g-1- **purothionin** (g-1P) from wheat, are known to inhibit insect [alpha]-amylase and may be toxic...

Description of the Invention:

15/3,KWIC/23 (Item 22 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3811293
Derwent Accession: 1994-183512
Utility
C/ **Biocidal proteins**
; **BACTERICIDES, FUNGICIDES**
Inventor: Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P. A., Alsemberg, BE
Rees, Sarah B., Forest Park, GB England
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB, England
Zeneca Ltd GB (Code: 32757)
Examiner: Hendricks, Keith D. (Art Unit: 184)
Law Firm: Cushman Darby & Cushman, L.L.P.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5597801	A	19970128	US 95451568	19950526
Division	Pending			US 93149839	19931110
CIP	Abandoned			US 932842	19930114
Priority				GB 9112300	19910607
				GB 9223708	19921112
				GB 933564	19930223

Fulltext Word Count: 14080

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed...

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...

...in 6M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...AMP1 nor Ac-AMP2 affected cell viability after 24 h of incubation. In contrast, [beta]-**purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/24 (Item 23 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3746704
Derwent Accession: 1993-100978
Utility
CM/ **Biocidal proteins**
; **ANTIFUNGAL OR ANTIBACTERIAL AGENTS**
Inventor: Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P. A., Alsemberg, BE
Osborn, Rupert W., Middlesex, GB England
Rees, Sarah B., Berkshire, GB England
Terras, Franky R. G., Amzegem, BE
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB, England
Zeneca Ltd GB (Code: 32757)
Examiner: Fox, David T. (Art Unit: 183)

supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well as...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens...

Non-exemplary or Dependent Claim(s):
...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/4 (Item 3 from file: 654)
DIALOG(R)File 654:US Pat.Full.

0004903757 **IMAGE Available
Derwent Accession: 1993-100978
Biocidal proteins
Inventor: Willem Broekaert, INV
Bruno Cammue, INV
Rupert Osborn, INV
Sarah Rees, INV
Franky Terras, INV
Jozef Vanderleyden, INV
Assignee: ZENECA Limited(03)
Correspondence Address: SYNGENTA CROP PROTECTION, INC., 2 RIGHTER PARKWAY P.O. BOX 15458, WILMINGTON, DE, 19850-5458, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20010014732	A1	20010816	US 2001759584	20010112
Division	US 5538525			US 95377687	19950125
Continuation	US 6187904			US 97971982	19971117
Continuation	US 5689043			US 95452078	19950526
Continuation	ABANDONED			US 932480	19930104
Continuation	UNKNOWN			WO 92GB1570	19920827
Priority				GB 9118523	19910829
				GB 923038	19920213
				GB 9213526	19920625

Fulltext Word Count: 14428

Summary of the Invention:
...Such proteins, including SI[small alpha, Greek]2 from sorghum and

[small gamma, Greek]-1- **purothionin** from wheat, are known to inhibit .insect [small alpha, Greek]-amylase and are toxic to...

Description of the Drawings:
...Lc-AFP, Ct-AMP1, sorghum SI[small alpha, Greek]2, wheat [small gamma, Greek]1 **purothionin** , and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:
...2PO[sub]4, 15 mM Na[sub]2HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...2PO[sub]4, 15 mM Na[sub]2HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [small gamma, Greek]- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in...small alpha, Greek]-amylase inhibitor SI[small alpha, Greek]2, wheat [small gamma, Greek]1 **purothionin** , and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...For the purpose of comparison, these tests were performed in parallel with [small beta, Greek]- **purothionin** , an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...with both test fungi. In comparison, the IC[sub]50 value of [small beta, Greek]- **purothionin**
(...Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [small beta, Greek]- **purothionin** administered at 50 [small mu, Greek]g/ml decreased the viability of both cell types...

Exemplary or Independent Claim(s):
...or bacteria which comprises exposure to SI[small alpha, Greek]2, [small gamma, Greek]-1- **purothionin** , or another [small alpha, Greek]-amylase inhibitor protein.

15/3,KWIC/5 (Item 4 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4547046
Derwent Accession: 1995-178646
Utility
CERTIFICATE OF CORRECTION
C/ **Imaging of colorectal cancer using ST receptor binding compounds ; VISUALIZING TUMOR CELLS; ADMINISTER RADIOACTIVE PARTICLES TO HUMANS AND DETECT POSITIONING AND ACCUMULATION OF RADIOACTIVE PARTICLES IN BODY**
Inventor: Waldman, Scott A., Ardmore, PA
Assignee: Thomas Jefferson University(02), Philadelphia, PA
Jefferson, Thomas University (Code: 06943)
Examiner: Celsa, Bennett (Art Unit: 168)
Assistant Examiner: Ricigliano, Joseph W.
Law Firm: Woodcock Washburn Kurt Mackiewicz & Norris LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6268159	A	20010731	US 98138237	19980821
Division	Pending			US 95468449	19950606
Division	US 5518888	A		US 93141892	19931026

Fulltext Word Count: 28331

Summary of the Invention:

...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

Description of the Invention:

...Compound 2-D14 comprises **purothionin** conjugated to SEQ ID NO:2... at room temperature in 0.4 M Tris-HCl, pH 8.0 and 1 mM **EDTA** . Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and...activity of the ST peptide. [sup]111 In is rapidly and potently chelated by either **EDTA** (**ethylenediaminetetraacetic** acid) or DTPA (**diethylenetriaminepetaacetic** acid). DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The [sup]111 In-DTPA is ...

15/3,KWIC/6 (Item 5 from file: 654)

4458415 **IMAGE Available
Derwent Accession: 1993-100978

Utility

C/ **Biocidal proteins**

; **AMINO ACID SEQUENCES OF MICROBIOCIDAL PROTEINS; FOR TRANSGENIC PLANTS WITH INCREASED DISEASE RESISTANCE**

Inventor: Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P. A., Alsemberg, BE
Osborn, Rupert W., Middlesex, GB
Rees, Sarah B., Berkshire, GB
Terras, Franky R. G., Amzegem, BE
Vanderleyden, Jozef, Heverlee, BE
Assignee: ZENECA Limited(03), London, GB
Zeneca Ltd GB (Code: 32757)
Examiner: Low, Christopher S. F. (Art Unit: 163)
Assistant Examiner: Gupta, Anish
Combined Principal Attorneys: Hohenschutz, Liza D.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6187904	A	20010213	US 97971982	19971117
Division	US 5538525	A		US 95377687	19950125
Continuation	US 5689043	A		US 95452078	19950526
Continuation	Abandoned			US 932480	19930104
Continuation	Abandoned			WO 92GB1570	19920827
Priority				GB 9118523	19910829
				GB 923038	19920213
				GB 9213526	19920625

Fulltext Word Count: 11346

Summary of the Invention:

...Biochem, 194:533-539). Such proteins, including SI[alpha]2 from sorghum and [gamma]-1- **purothionin** from wheat, are known to inhibit insect [alpha]-amylase and are toxic to insect larvae...

Description of the Drawings:
...AMP1, the Cb-AMPs, Lc-AFP, Ct-AMP1, sorghum SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored ...Sorghum bicolor (Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [gamma]-**purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in...Lc-AFP, Ct-AMP1, the sorghum [alpha]-amylase inhibitor SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...supplement, respectively. For the purpose of comparison, these tests were performed in parallel with [beta]-**purothionin** , an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...30-fold with both test fungi. In comparison, the IC[sub]50 value of [beta]- **purothionin**
(...AFP2, nor Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml

15/3,KWIC/7 (Item 6 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4458048
Derwent Accession: 1998-456873
Utility
CERTIFICATE OF CORRECTION
C/ **Methods of identifying and detecting pancreatic cancer ; IN VITRO DIAGNOSIS OF TUMORS IN HUMANS; ANALYZING PREFERENTIAL ORGAN TISSUE FOR PRESENCE OF CHOLECYSTOKININ MESSENGER RIBONUCLEIC ACIDS, PRESENCE OF CHOLECYSTOKININ MESSENGER RIBONUCLEIC ACIDS INDICATES HUMAN HAS A TUMOR**
Inventor: Weinberg, David, Philadelphia, PA
Waldman, Scott A., Ardmore, PA
Barber, Michael T., Paoli, PA
Biswas, Sanjoy, Philadelphia, PA
Assignee: Thomas Jefferson University(02), Philadelphia, PA
Jefferson, Thomas University (Code: 06943)
Examiner: Eyler, Yvonne (Art Unit: 162)
Assistant Examiner: Holleran, Anne L.
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6187536	A	20010213	US 9825534	19980218
Provisional				US 60-38063	19970218

Fulltext Word Count: 23168

Summary of the Invention:

...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well as ...

15/3,KWIC/8 (Item 7 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4427576
Derwent Accession: 2000-687452
Utility
C/ **X-ray guided drug delivery**
; **TARGETING A TISSUE; EXPOSURE OF TISSUE TO IONIZING RADIATION**
Inventor: Hallahan, Dennis E., Nashville, TN
Assignee: Vanderbilt University(02), Nashville, TN
Vanderbilt University (Code: 88418)
Examiner: Schwartzman, Robert A. (Art Unit: 166)
Assistant Examiner: Sandals, William
Law Firm: Jenkins & Wilson, P.A.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6159443	A	20001212	US 99302456	19990429

Fulltext Word Count: 26146

Summary of the Invention:
...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin...

Description of the Invention:
...by staining for factor VIII. Confluent cells were harvested with 0.1% collagenase 0.01% **EDTA** and subcultured at a ratio of 1:3. HUVECs were used at third passage;

Non-exemplary or Dependent Claim(s):
...4 fluorouracil, melphalan, chlorambucil, a nitrogen mustard, cyclophosphamide, cis-platinum, vindesine, vinca alkaloids, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin, doxorubicin, daunomycin, vinblastine...daunorubicin, cytosine arabinoside, etoposide, 5-4

4317418
Derwent Accession: 1995-178646
Utility
C/ **Compositions that specifically bind to colorectal cancer cells and methods of using the same**
; **CONJUGATED COMPOUNDS WHICH COMPRISE AN ST RECEPTOR BINDING MOIETY AND A RADIOSTABLE ACTIVE MOIETY**
Inventor: Waldman, Scott A., Ardmore, PA
Assignee: Thomas Jefferson University(02), Philadelphia, PA
Jefferson, Thomas University (Code: 06943)
Examiner: Brusca, John S. (Art Unit: 165)
Assistant Examiner: Larson, Thomas G.
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6060037	A	20000509	US 96635930	19960426
CIP	US 5518888	A		US 93141892	19931026
PCT	WO 9511694		19950504	WO 94US12232	19941026
			371:19960426		
			102e:19960426		
	US 5601990	A		US 94305056	19940913

Fulltext Word Count: 39301

Description of the Invention:
...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1, ... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well asCompound 2-D14 comprises **purothionin** conjugated to SEQ ID NO:2...at room temperature in 0.4 M Tris-HCl, pH 8.0 and 1 mM **EDTA** . Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and...activity of the ST peptide. [sup]111 In is rapidly and potently chelated by either **EDTA** (**ethylenediaminetetraacetic** acid) or DTPA (**diethylenetriaminepetaacetic** acid) . DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The [sup]111 In-DTPA is ...

15/3,KWIC/11 (Item 10 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4239629
Derwent Accession: 1994-249225
Utility
REISSUE REQUESTED **See File 123 for details
C/ **High lysine derivatives of [alpha]-hordothionin ; FOR TRANSFORMED PLANTS AND CELLS WITH ENHANCED LYSINE CONTENT AND WHICH SUPPRESS AND KILL PLANT PATHOGENS INCLUDING FUNGI**
Inventor: Rao, A. Guruaj, Urbandale, IA
Beach, Larry, Des Moines, IA
Assignee: Pioneer Hi-Bred International, Inc.(02), Des Moines, IA
Pioneer Hi-Bred International Inc (Code: 17947)
Examiner: Smith, Lynette R. F. (Art Unit: 169)
Assistant Examiner: Kimball, Melissa L.
Law Firm: Pioneer Hi-Bred International, Inc.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5990389	A	19991123	US 97838763	19970410
Continuation	Abandoned			US 95575654	19951220
Continuation	Abandoned			US 95369975	19950106
Continuation	Abandoned			US 933885	19930113

Fulltext Word Count: 4619

Description of the Invention:
...crystal structures have not previously been available for hordothionin or even related compounds such as **purothionin** and viscotoxin. We undertook to develop such structural information...oil, corn oil and soybean oil; polyols such as propylene glycol, glycerin, sorbitol, mannitol and **polyethylene** glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium...

15/3,KWIC/12 (Item 11 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4235015
Derwent Accession: 1994-183512
Utility
C/ **Transgenic plants expressing biocidal proteins ; ISOLATED ANTIMICROBIAL PROTEIN HAVING AMINO ACID SEQUENCECONTAINING COMMON CYSTEINE/GLYCINE DOMAIN OF CHITIN BINDINGPLANT PROTEINS; AGICULTURAL AND PHARMACEUTICAL APPLICATIONS AS FUNGICIDES OR BACETRICIDES**
Inventor: Broekaert, Willem Frans, Dilbeek, BE
Cammue, Bruno Phillippe Angelo, Alsemberg, BE
Rees, Sarah Bronwen, Forest Park, GB
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB
Zeneca Ltd GB (Code: 32757)
Examiner: Kemmerer, Elizabeth (Art Unit: 166)
Combined Principal Attorneys: Hohenschutz, Liza D.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5986176	A	19991116	US 96777113	19961230
Division	US 5691199	A		US 95451566	19950526
Division	US 5514779	A		US 93149839	19931110
CIP	Abandoned			US 932842	19930114
CIP	Pending			WO 92GB999	19920603
Priority				GB 9112300	19910607
				GB 9223798	19921112
				GB 933564	19930223

Fulltext Word Count: 13574

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed... sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...AMP1 nor Ac-AMP2 affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/13 (Item 12 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4187951
Derwent Accession: 1992-331736
Utility
C/ **Biocidal proteins**
; **TRANSFORMED BIOLOGICAL PROTEIN**
Inventor: De Bolle, Miguel, Leuven, BE
Broekaert, Willem Frans, Dilbeek, BE
Cammue, Bruno Philippe Angelo, Alsemberg, BE
Rees, Sarah Bronwen, Bracknell, GB
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB
Zeneca Ltd GB (Code: 32757)
Examiner: Fox, David T. (Art Unit: 169)
Combined Principal Attorneys: Hohenschutz, Liza D.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5942663	A	19990824	US 97915142	19970820
Division	US 5482928	A		US 93117080	19931220
Continuation	US 5689048	A		US 95471329	19950602
Priority				GB 915052	19910311
				GB 915684	19910319
				WO 92GB423	19920310

Fulltext Word Count: 6005

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The ...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithiothreitol (DTT). Silver staining...dioica agglutinin or UDA (Broekaert, WF et al; 1989; Science, 245, 1100-1102) and [beta]- **purothionin** (Hernandez-Lucas, C et al; 1974; Appl Microbiol, 28, 165-168). Fungi were grown on...

...as previously described (Peumans, WJ et al; 1983; FEBS Lett, 177, 99-103). The [beta]- **purothionin** was purified from wheat endosperm by the method of Redman, DG and Fisher, N (1969...Table 2 summarises the results. Serial dilutions of Mj-AMP1, Mj-AMP2, UDA and [beta]- **purothionin** were applied to fungi and the percent growth inhibition measured by microspectrophotometry (as described in...
...g/ml for Mj-AMP2, from 0.5 to 15 [mu]g/ml for [beta]- **purothionin** , and

from 20 to over 1,000 [mu]g/ml for UDA depending on the...

...On an average basis the obtained antifungal activity series is as follows: Mj-AMP2=[beta]- **purothionin** >Mj-AMP1>UDA. Some fungi, such as B cinerea, C lindemuthianum and V inaequalis, are clearly more sensitive to Mj-AMP2 than to [beta]- **purothionin** . Conversely, the latter protein is most effective in deterring growth of other fungi such as... time-dependent drop in antifungal activity, however, was less pronounced for Mj-AMP2 and [beta]- **purothionin** than for Mj-AMP1 or UDA. Also, Mj-AMP2 and [beta]- **purothionin** characteristically produced steeper dose-response curves than Mj-AMP1 or UDA. FIG. 4 shows the...

...and B), Mj-AMP2 (panels C and D), UDA (panels E and F), and [beta]- **purothionin** (panels G and H). The percent growth inhibition was recorded after 48 h (.circle-solid...positive and gram-negative bacteria: Bacillus megaterium, Sarcina lutea, Escherichia coli and Erwinia carotovora. [beta]- **purothionin** and UDA were also tested for comparison (see Example 7). Tests were performed in soft...

15/3,KWIC/14 (Item 13 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4163914
Derwent Accession: 1995-246394
Utility
C/ **Transformed plants expressing antimicrobial proteins ; ANTIMICROBIAL PROTEIN CAPABLE OF ISOLATION FROM SEEDS OF HEUCHERA OR AESCULUS**
Inventor: Broekaert, Willem Frans, Dilbeek, BE
Cammue, Bruno Philippe Angelo, Alsemberg, BE
Osborn, Rupert William, Middlesex, GB
Rees, Sarah Bronwen, Bracknell, GB
Assignee: Zeneca Limited(03), London, GB
Zeneca Ltd GB (Code: 32757)
Examiner: Robinson, Douglas W. (Art Unit: 169)
Assistant Examiner: Zaghmout, Ousama M-Faiz
Combined Principal Attorneys: Hohenschutz, Liza D.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5919918	A	19990706	US 97956459	19971023
Division	Pending			US 656318	
Priority				GB 9326424	19931224

Fulltext Word Count: 5637

Summary of the Invention:
...Biochem, 194:533-539). Such proteins, including Si[alpha]2 from sorghum and g-1- **purothionin** (g-1P) from wheat, are known to inhibit insect [alpha]-amylase and may be toxic...

Description of the Invention:
...sub]4, 15 mm Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Two hundred...

15/3,KWIC/15 (Item 14 from file: 654)
DIALOG(R)File 654:US Pat.Full.

QUICKENDEN TI, 1981, V85, P2232, J PHYS CHEM-US
QUICKENDEN TI, 1975, V17, P147, SOL ENERGY
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RABINOWITCH E, 1940, V8, P551, J CHEM PHYS
RABINOWITCH E, 1940, V8, P560, J CHEM PHYS
RABINOWITCH E, 1940, V4, P193, SOL ENERGY
RIDEAL EK, 1925, V127, P258, J CHEM SOC
ROTHAGHIMUKHERJ.KK, 1983, V31, P417, SOL ENERGY
SAKATA T, 1977, V81, P537, J PHYS CHEM-US
SHIGEHARA K, 1978, V23, P855, ELECTROCHIM ACTA
SHIGEHARA K, 1977, V81, P1883, J PHYS CHEM-US
SUDA Y, 1978, V82, P268, J PHYS CHEM-US
TIEN HT, 1978, V125, P885, J ELECTROCHEM SOC
TRAMLER G, 1915, V90, P385, Z PHYS CHEM
WILDES PD, 1975, V97, P2288, J AM CHEM SOC
WILDES PD, 1978, V100, P6568, J AM CHEM SOC
WILDES PD, 1978, V82, P981, J PHYS CHEM-US
WILDES PD, 1977, V19, P567, SOL ENERGY
WILDES PD, 1978, V19, P597, SOL ENERGY
WRIGHTON MS, 1979, V12, P303, ACCOUNTS CHEM RES

11/9/7 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02513289 Genuine Article#: LG689 Number of References: 0
(NO REFS KEYED)
Title: USE OF TRITON X-100 IN A PHOTOGALVANIC CELL FOR SOLAR-ENERGY CONVERSION AND STORAGE - THIONINE EDTA **SYSTEM**
Author(s): AMETA SC; CHITTORA AK; KHAMESRA S; AMETA R
Corporate Source: SUKHADIA UNIV,UNIV COLL SCI,DEPT CHEM,15 RADHEYSHYAM ST,BHRAMPOLE GATE/UDAIPUR 313001//INDIA/
Journal: ARABIAN JOURNAL FOR SCIENCE AND ENGINEERING, 1992, V17, N4A (OCT), P477-480
ISSN: 0377-9211
Language: ENGLISH Document Type: ARTICLE
Geographic Location: INDIA
Subfile: SciSearch; CC ENGI--Current Contents, Engineering, Technology & Applied Sciences
Journal Subject Category: MULTIDISCIPLINARY SCIENCES
Abstract: **Thionine , ethylenediaminetetraacetic** acid, and Triton X-100 (Tx) have been used as photosensitizer, reductant, and surfactant, respectively in a photogalvanic cell for solar energy conversion. The photocurrent and photopotential generated by this cell were 70.0 muA and 888.0 mV, respectively. The effect of various parameters on the electrical output of the cell was studied, and current-voltage characteristics of the cell have also been observed.

11/9/10 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

08896137 PASCAL No.: 90-0064116
Use of the thionine **- EDTA system in photogalvanic cells for solar energy conversion**
AMETA S C; SADHNA KHAMESRA; SUSHILA LODHA; RAMESHWER AMETA
Sukhadia univ., dep. chemistry, Udaipur 313 001, India
Journal: Journal of photochemistry and photobiology. A. Chemistry, 1989, 48 (1) 81-86
ISSN: 531731 CODEN: JPPCEJ Availability: CNRS-15990A
No. of Refs.: 19 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: Switzerland
Language: English
Description d'une cellule solaire utilisant de la **thionine** comme

Bowman-Birk...

...difference, other conditions were as in Fig. 6.
Reduction After
Protein 20 min 120 min
Purothionin -P 15 32
DSG-1 22 38
Corn kernel trypsin
inhibitor 3 15
Bowman-Birk...

...CM-1 a-amylase inhibitors (147 and 210%,,
respectively) ; corn kernel trypsin inhibitor (424%-) ; and
purothionin (82, 133, and 120% for the a,, al and #
forms, respectively) , Glutaredoxin was inef f...and
ovomucoid inhibitor) . Those proteins that were reduced
by either thioredoxin or glutaredoxin include the
purothionins , two a-amylase inhibitors (DSG-1, CM-1),
a cystine-rich trypsin inhibitor from plants...0.1 ml of 20
mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA**
at 300C for 2 hours. The concentrations of thioredoxin,
NTR,, and NADPH were 0,024 mg/mIj, 0,02 mg/ml, and 0.25
mM, respectively. With DTT as reductant,, **EDTA** and
components of the NADP/thioredoxin system were omitted.
Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HC1, pH
7.5, containing 200
mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase
inhibitor protein was purified as described below.

CM32 Chromatography
The pullulanase inhibitor sample...

...30 mM Tris
HC11 pH 7,5, containing 200 mM Na Cl and 1 mM **EDTA** ,
Fractions (3*6 ml/fraction) showing pullulanase
inhibitory activity were pooled, concentrated by
dialysis against...

15/3,KWIC/58 (Item 27 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00230900
BIOCIDAL PROTEINS
PROTEINES BIOCIDES
Patent Applicant/Assignee:
IMPERIAL CHEMICAL INDUSTRIES PLC,
BROEKAERT Willem Frans,
CAMMUE Bruno Philippe Angelo,
OSBORN Rupert William,
REES Sarah Bronwen,
TERRAS Franky Raymond Gerard,
VANDERLEYDEN Jozef,
Inventor(s):
BROEKAERT Willem Frans,
CAMMUE Bruno Philippe Angelo,
OSBORN Rupert William,
REES Sarah Bronwen,
TERRAS Franky Raymond Gerard,
VANDERLEYDEN Jozef,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9305153 A1 19930318
Application: WO 92GB1570 19920827 (PCT/WO GB9201570)
Priority Application: GB 9118523 19910829; GB 923038 19920213; GB 9213526 19920625
Designated States:

(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AU BB BG BR CA CS FI HU JP KP KR LK MG MN MW NO PL RO RU SD US AT BE CH DE DK ES FR GB GR IE IT LU MC NL SE BF BJ CF CG CI CM GA GN ML MR SN TD TG
Publication Language: English
Fulltext Word Count: 13654

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... 1990
Eur J Biochem, 194:533-539). Such proteins, including SI=2 from sorghum andy **purothionin** from wheat, are known to inhibit insect a-amylase and are toxic to insect larvae...Rs-AFPI, Dm-AMP1. the Cb-AMPs, Lc-AFP, Ct-AMPI, sorghum Sia2, wheat Y1 **purothionin** , and the predicted products of the pea genes p1230 and pI39, of the cowpea gene...buffer containing 10 mM NaH2PO VP 15,mM Na2HPO 4J' 100 mm KC11 2 mM **EDTA** , 2 mK thiourea, and 1 mN PMSF. The homogenate was squeezed through cheesecloth and clarified...NaH PO 15 mM Na HPO 100 mm 2 41 2 41 KClr 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HC1 (pH 8,3), 1% (w/v) SDS, 1 mM **EDTA** , 0,005% bromophenol blue and, unless otherwise stated, 1% (w/v) dit loerythritol (DTE).

Proteins...6 M guanidinium-Cl containing 100 mm sodium phosphate buffer (pH 7) and 1 mM **EDTA** , The mixtures were allowed to react with 5,5t-dithionitrobenzoic acid and monitored for release...Sorghum bicolor (Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to y- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit 'in...

...the Cb-AMPs, Lc-AFP, Ct-AMP1, the sorghum a-amylase inhibitor SIa2, wheat yl **purothionin** , and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...supplement, respectively, For the purpose of comparison, these tests were performed in parallel with 0- **purothionin** , an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...

...more than 30-fold with both test fungi, in comparison, the IC50 value of 0- **purothionin** TABLE 6 VARIATIONS IN ANTIFUNGAL ACTIVITY IN THE PRESENCE OF X+ AND Fungus Antifungal IC50...

...6 10
Rs-AFP2 3 2 2 2
Rs-nsLTP 20 35 >1000 108
0- **purothionin** 10 7 4 10
Mj-AMP2 4 5 40 50
T hamatum Rs-AFPl 7...

...7 30

FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	200133	408
CLAIMS B	(German)	200133	344
CLAIMS B	(French)	200133	431
SPEC B	(English)	200133	5803
Total word count - document A			0
Total word count - document B			6986
Total word count - documents A + B			6986

...SPECIFICATION buffer containing 10 mM NaH2))PO4)), 15 mM Na2))HPO4)), 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithiothreitol (DTT). Silver staining...dioica agglutinin or UDA (Broekaert, WF et al; 1989; Science, 245, 1100-1102) and (beta)- **purothionin** (Hernandez-Lucas, C et al; 1974; Appl Microbiol, 28, 165-168). Fungi were grown on...as previously described (Peumans, WJ et al; 1983; FEBS Lett, 177, 99-103). The (beta)- **purothionin** was purified from wheat endosperm by the method of Redman, DG and Fisher, N (1969...

...Table 2 summarises the results. Serial dilutions of Mj-AMP1, Mj-AMP2, UDA and (beta)- **purothionin** were applied to fungi and the percent growth inhibition measured by microspectrophotometry (as described in...

...g/ml for Mj-AMP2, from 0.5 to 15 (mu)g/ml for (beta)- **purothionin** , and from 20 to over 1,000 (mu)g/ml for UDA depending on the...

...On an average basis the obtained antifungal activity series is as follows: Mj-AMP2 = (beta)- **purothionin** > Mj-AMP1 > UDA. Some fungi, such as B cinerea, C lindemuthianum and V inaequalis, are clearly more sensitive to Mj-AMP2 than to (beta)- **purothionin** . Conversely, the latter protein is most effective in deterring growth of other fungi such as...

...time-dependent drop in antifungal activity, however, was less pronounced for Mj-AMP2 and (beta)- **purothionin** than for Mj-AMP1 or UDA. ALso, Mj-AMP2 and (beta)- **purothionin** characteristically produced steeper dose-response curves than Mj-AMP1 or UDA. Figure 5 shows the...

...and B), Mj-AMP2 (panels C and D), UDA (panels E and F), and (beta)- **purothionin** (panels G and H). The percent growth inhibition was recorded after 48 h (*----*), after 60...positive and gram-negative bacteria: Bacillus megaterium, Sarcina lutea, Escherichia coli and Erwinia carotovora. (beta)- **purothionin** and UDA were also tested for comparison (see Example 7). Tests were performed in soft...

15/3,KWIC/67 (Item 3 from file: 348)

00537586

Natural and synthetic proteins with inhibitory activity towards pathogenic microorganisms.

Naturliche und synthetische Proteine mit inhibitorischer Aktivitat gegen pathogene Mikroorganismen.

Proteines naturelles et synthetiques avec activite inhibitrice contre des microorganismes pathogenes.

PATENT ASSIGNEE:

PIONEER HI-BRED INTERNATIONAL, INC., (1142071), 700 Capital Square 400 Locust Street, Des Moines Iowa 50309, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IT;LI;LU;NL;SE)

INVENTOR:

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LEGAL REPRESENTATIVE:
Göldin, Douglas Michael et al (31062), J.A. KEMP & CO. 14, South Square Gray's Inn, London WC1R 5LX, (GB)
PATENT (CC, No, Kind, Date): EP 502718 A1 920909 (Basic)
APPLICATION (CC, No, Date): EP 92301868 920304;
PRIORITY (CC, No, Date): US 664270 910304
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; NL; SE
INTERNATIONAL PATENT CLASS: A01N-037/46; A01N-037/44; A01N-063/00; A01N-065/00; C12N-015/00; C12N-015/52;
ABSTRACT WORD COUNT: 54

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	959
SPEC A	(English)	EPABF1	5135
Total word count - document A			6094
Total word count - document B			0
Total word count - documents A + B			6094

...SPECIFICATION oil, corn oil and soybean oil; polyols such as propylene glycol, glycerin, sorbitol, mannitol and **polyethylene** glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium...hormone 1-24, Citrate synthase, Defensin NP1, Cathepsin G, Lysozyme, a-hordothionin, b-hordothionin, b- **purothionin** , Crotamine, Melittin, Eosinophil major basic protein, Eosinophil cationic protein, and Eosinophil peroxidase.
Preferred proteins for...

...hormone 1-24, Citrate synthase, Defensin NP1, Cathepsin G, Lysozyme, a-hordothionin, b-hordothionin, b- **purothionin** , Melittin, Eosinophil major basic protein, Eosinophil cationic protein, and Eosinophil peroxidase.
Preferred proteins for killing...

...L-Lysine HCl, poly-D-Lysine, poly-D-Lysine HBr, Defensin NP1, a-hordothionin, b- **purothionin** and Melittin.
Preferred proteins for killing or inhibiting the pathogen Colletototrichum graminicola are: Magainin-A...

...L-Lysine HCl, poly-D-Lysine, poly-D- Lysine HBr, Defensin NP1, a-hordothionin, b- **purothionin** and Melittin.
Preferred proteins for killing or inhibiting the pathogen Verticillium albo-atrum are: Magainin...

...L-Lysine HCl, poly-D-Lysine, poly-D-Lysine HBr, Defensin NP1, a-hordothionin, b- **purothionin** and Melittin.
Preferred proteins for killing or inhibiting the pathogen Phytophthora megaspermae f.sp. glycinea are: Magainin-2, poly-L-Lysine HBr, Defensin NP1, b- **purothionin** and Melittin.
Preferred proteins for killing or inhibiting the pathogen Macrophomina phaseolina are: poly-L-histidine, poly-D-Lysine, poly-D-Lysine HBr, Defensin NP1, a-hordothionin and b- **purothionin** .
Preferred proteins for killing or inhibiting the pathogen Diaporthe phaseolorum caulivora are: Defensin NP1, a-hordothionin and b- **purothionin** .
Preferred proteins for killing or inhibiting the pathogen Sclerotinia sclerotiorum are: Magainin-A, Magainin-G...

...poly-D-Lysine HBr, Mastoparan, Defensin NP1, Cathepsin G, Lysozyme, a-hordothionin, b-hordothionin, b- **purothionin** , stinging nettle lectin, Crotamine, Melittin, Eosinophil major basic protein and Eosinophil cationic protein.
Preferred proteins...

Previous Doc Next Doc Go to Doc#
First Hit

Generate Collection

L5: Entry 27 of 28 File: DWPI May 15, 2003

DERWENT-ACC-NO: 2003-585564
DERWENT-WEEK: 200355

TITLE: Bacterial composition used for sterilizing food poisoning bacteria, includes ethylenediaminetetraacetic acid or its metal salts and alpha- or beta-type thionin

Basic Abstract Text (1):
NOVELTY - A bacterial composition comprises EDTA or its metal salts and alpha - or beta -type thionin.

Previous Doc Next Doc Go to Doc#

Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

The usefulness of **thionin** for staining cartilage sections embedded in glycol methacrylate (GMA) and the effect of decalcification on cartilage sections embedded in paraffin and GMA were assessed. Short decalcification periods using 5% formic acid or 10% **EDTA** did not influence the staining properties or the morphology of cartilage matrix and chondrocytes. The standard stain safranin O-fast green for differential staining of cartilage was used as control in these experiments. Prolonged exposure of safranin O stained sections to fast green resulted in disappearance of the safranin O stained matrix, thereby hampering the quantitative measurement of negatively charged glycosaminoglycans (GAG). **Thionin** stained evenly throughout all cartilage layers, independent of the staining times. In contrast to safranin O, **thionin** did not show metachromasia in nondehydrated cartilage sections, which made it more suitable for assessing cartilage quality in GMA embedded cartilage. To evaluate the selectivity of **thionin** staining in cartilage, chondroitinase ABC and trypsin digestions were carried out. **Thionin** staining was prevented by these enzymes in the territorial matrix, representing the interlacunar network and the chondrocyte capsule. Staining with **thionin** of the interterritorial matrix was only slightly reduced, possibly representing keratan sulfate and hyaluronic acid in cartilage of elderly patients. Comparison of **thionin** stained GMA embedded cartilage with safranin O stained paraffin embedded sections showed significant similarity in optical densitometry, indicative of the specificity of **thionin** bound to negatively charged GAG in cartilage. In GMA embedded cartilage morphology was relatively intact compared to paraffin embedded sections due to less shrinkage of chondrocytes and the interlacunar network.

Tags: Comparative Study
Descriptors: *Cartilage, Articular--cytology--CY; *Methacrylates; *Paraffin Embedding; *Phenothiazines--metabolism--ME; Aged; Cartilage, Articular--anatomy and histology--AH; Chondroitin Lyases--metabolism--ME; Decalcification Technique; Glycosaminoglycans--metabolism--ME; Humans; Phenazines; Plastic Embedding; Sensitivity and Specificity; Staining and Labeling--methods--MT
CAS Registry No.: 0 (Glycosaminoglycans); 0 (Methacrylates); 0 (Phenazines); 0 (Phenothiazines); 477-73-6 (safranine T); 581-64-6 (thionine); 868-77-9 (hydroxyethyl methacrylate)
Enzyme No.: EC 4.2.2.- (Chondroitin Lyases)
Record Date Created: 19930412
Record Date Completed: 19930412

11/9/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05131256 PMID: 594487
[Selective photooxidation of methionine, tyrosine and tryptophane using thionine **and toluidine blue as sensitizers (author's transl)]**
Fotooxidacion selectiva de metionina, tirosina y triptofano utilizando tionina y azul de toluidina como fotosensibilizadores.
Iborra J L; Llorca F I; Pastor R F; Garcia J V
Revista espanola de fisiologia (SPAIN) Dec 1977, 33 (4) p297-304, ISSN 0034-9402 Journal Code: 0404475
Publishing Model Print
Document type: Journal Article ; English Abstract
Languages: SPANISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Thionine and toluidine blue were used as sentizers on photooxidation processes of methionine, tryosine and tryptophane. They were more effective

than methylene blue. Methionine was photooxidized to sulfoxide and tryptophane to kinurenine. A tyrosine-sensitizer addition compound was postulated. Dye concentration, pH, temperature and **EDTA** presence conditions were determined on each one of the modification reactions. Methionine at acid pH was selectively modified. On the basis of obtained results and published references, a direct interaction of singlet oxygen with methionine and tryptophase and the excited dye with tyrosine was respectively discussed.
Tags: Comparative Study
Descriptors: *Amino Acids--metabolism--ME; *Photosynthesis--drug effects --DE; *Tyrosine--metabolism--ME; Ergothioneine--pharmacology--PD; Methionin e--metabolism--ME; Oxidation-Reduction--drug effects--DE; Photic Stimulation; Tolonium Chloride--pharmacology--PD; Tryptophan--metabolism --ME
CAS Registry No.: 0 (Amino Acids); 497-30-3 (Ergothioneine); 55520-40-6 (Tyrosine); 63-68-3 (Methionine); 73-22-3 (Tryptophan); 92-31-9 (Tolonium Chloride)
Record Date Created: 19780223
Record Date Completed: 19780223

11/9/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04488011 PMID: 806084
Mechanism of photoreduction of thiazine dyes by EDTA **studied by flash photolysis III. Consequences of a newly found pKT of** thionine **on the mechanism in basic solutions.**
Bonneau R; Pereyre J
Photochemistry and photobiology (ENGLAND) Mar 1975, 21 (3) p173-7, ISSN 0031-8655 Journal Code: 0376425
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Descriptors: *Dyes; *Edetic Acid; *Light; *Oxidation-Reduction; *Phenothiazines; *Photolysis; *Thiazines--metabolism--ME; Imines
CAS Registry No.: 0 (Dyes); 0 (Imines); 0 (Phenothiazines); 0 (Thiazines); 60-00-4 (Edetic Acid)
Record Date Created: 19750829
Record Date Completed: 19750829

11/9/5 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0003236407 BIOSIS NO.: 198171055366
CHARACTERIZATION OF THE RED LIGHT INDUCED REDUCTION OF A PARTICLE ASSOCIATED B TYPE CYTOCHROME FROM CORN IN THE PRESENCE OF METHYLENE BLUE
AUTHOR: WIDELL S (Reprint); BRITZ S J; BRIGGS W R
AUTHOR ADDRESS: DEP PLANT PHYSIOL, BOX 7007, S-22007, LUND 7, SWEDEN** SWEDEN
JOURNAL: Photochemistry and Photobiology 32 (5): p669-678 1980
ISSN: 0031-8655
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Methylene blue transfers electrons to a membrane-associated b-type cytochrome in particulate fractions from corn coleoptiles. The Km for methylene blue is less than 1 .mu.M under optimal conditions. This reaction is destroyed by boiling, but not by 7 M urea. Kinetic analyses of the influence of light intensity on cytochrome reduction suggest that

a 1st order photochemical reaction is limiting. Free **EDTA** may serve as an electron donor in this system at least at high methylene blue and protein concentrations. The photoactivity does not coincide either with mitochondrial or endoplasmic reticulum markers, and may be localized in plasma membrane. There is an estimated 5 .times. 10-10 mol photoreducible cytochrome/g coleoptile tissue. Studies on the effect of pH on the reaction in the presence of methylene blue or **thionine** indicate that dye photoreduction itself is not rate-limiting. Wavelength dependence studies suggest that it is methylene blue monomer and not dimer which mediates the reaction. Although O2 is apparently required for the reaction, neither superoxide nor excited singlet oxygen appear to be involved. The reaction mechanism is still unknown.

REGISTRY NUMBERS: 61-73-4: METHYLENE BLUE; 60-00-4: **EDTA** ; 7782-44-7: OXYGEN
DESCRIPTORS: COLEOPTILES ELECTRON TRANSFER **EDTA** WAVELENGTH DEPENDENCE OXYGEN REQUIREMENT
DESCRIPTORS:
MAJOR CONCEPTS: Bioenergetics--Biochemistry and Molecular Biophysics; Enzymology--Biochemistry and Molecular Biophysics; Radiation Biology
BIOSYSTEMATIC NAMES: Gramineae--Monocotyledones, Angiospermae, Spermatophyta, Plantae
COMMON TAXONOMIC TERMS: Angiosperms; Monocots; Plants; Spermatophytes; Vascular Plants
CHEMICALS & BIOCHEMICALS: METHYLENE BLUE; **EDTA** ; OXYGEN
CONCEPT CODES:
10012 Biochemistry - Gases
10060 Biochemistry studies - General
10064 Biochemistry studies - Proteins, peptides and amino acids
10065 Biochemistry studies - Porphyrins and bile pigments
10510 Biophysics - Bioenergetics: electron transport and oxidative phosphorylation
10604 External effects - Light and darkness
10802 Enzymes - General and comparative studies: coenzymes
51516 Plant physiology - Light and radiation effects
52504 Agronomy - Grain crops
BIOSYSTEMATIC CODES:
25305 Gramineae

11/9/8 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

01233933 Genuine Article#: GG799 Number of References: 91
Title: MODEL PHOTOGALVANIC SYSTEMS FOR THE CONVERSION OF SOLAR-ENERGY
Author(s): SHAGISULTANOVA GA; TAITS AM; TIMONOV AM
Corporate Source: AI GERTSEN STATE TEACHERS INST/LENINGRAD//USSR/
Journal: JOURNAL OF APPLIED CHEMISTRY OF THE USSR, 1990, V63, N12, P 2427-2440
Language: ENGLISH Document Type: ARTICLE
Geographic Location: UNION OF SOVIET SOCIALIST REPUBLICS
Subfile: SciSearch; CC PHYS--Current Contents, Physical, Chemical & Earth Sciences
Journal Subject Category: CHEMISTRY, APPLIED
Identifiers--KeyWords Plus: **THIONINE** -COATED ELECTRODE; **ETHYLENEDIAMINETETRAACETIC** ACID SYSTEM; PHOTO-CHEMICAL MECHANISMS; PHOTOELECTROCHEMICAL CELL; METHYLENE-BLUE; RATE CONSTANT; IRON SYSTEM; POWER; EFFICIENCY; KINETICS
Research Fronts: 89-6804 002 (PHOTOGALVANIC CELL FOR SOLAR-ENERGY CONVERSION; PHENOSAFRANIN DYE; METHYLENE BLUE-NITRILOTRIACETIC ACID SYSTEM)
Cited References:
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ALBERY WJ, 1980, P341, FARADAY DISCUSS CHEM

ALBERY WJ, 1981, V5, P794, J CHEM SOC P2
ALBERY WJ, 1978, V86, P1, J ELECTROANAL CHEM
ALBERY WJ, 1978, V86, P19, J ELECTROANAL CHEM
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AMETA SC, 1979, V48, P81, J PHOTOCHEM PHOTOBIO
AMETA SC, 1985, V266, P20, Z PHYS CHEM
AMETA SC, 1986, V267, P1230, Z PHYS CHEM
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KAMAT PV, 1984, V131, P1032, J ELECTROCHEM SOC
KAMAT PV, 1981, V85, P1474, J PHYS CHEM-US
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KIRSCHDEMESMAEK.AO, 1979, V3, P239, NOUV J CHIM
LICHTIN NN, 1979, P296, ADV CHEM RES
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MATHAI KG, 1962, V68, P683, J PHYS CHEM-US
MEMMING P, 1988, V143, P79, TOP CURR CHEM
MOUNTZ JM, 1978, V21, P291, SOL ENERGY
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MURTHY ASN, 1979, V3, P205, INT J ENERG RES
MURTHY ASN, 1980, V4, P339, INT J ENERG RES
MURTHY ASN, 1983, V30, P39, SOL ENERGY
NEUMANSPALLART M, 1982, V86, P2681, J PHYS CHEM-US
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NOZIK AJ, 1980, V295, P453, PHILOS T R SOC LON A
OSIF TL, 1978, V82, P1778, J PHYS CHEM-US
PRAJUNTABORIBAL K, 1987, V38, P149, SOL ENERGY
QUICKENDEN TI, 1978, V23, P1045, ELECTROCHIM ACTA
QUICKENDEN TI, 1979, V24, P143, ELECTROCHIM ACTA
QUICKENDEN TI, 1985, V132, P81, J ELECTROCHEM SOC
QUICKENDEN TI, 1979, V83, P2496, J PHYS CHEM-US
QUICKENDEN TI, 1980, V84, P870, J PHYS CHEM-US

photosensibilisant et l' **EDTA** comme reducteur: caracteristiques potentiometriques en fonction de la concentration en **thionine** , en **EDTA** , du pH et de la geometrie de la couche et mecanismes de reaction.

English Descriptors: Solar cell; Energy conversion; Electrical characteristic; Photosensitizer; Medium effect; Potentiometry; Chemical concentration; Experimental study; Reaction mechanism; **EDTA**
French Descriptors: Cellule solaire; Conversion energie; Caracteristique electrique; Photosensibilisant; Effet milieu; Potentiometrie; Concentration chimique; Etude experimentale; Mecanisme reaction; **EDTA** ; **Thionine**

Classification Codes: 230C02B; 001D06C02

11/9/11 (Item 2 from file: 144)

05746232 PASCAL No.: 84-0247280
Electrochemistry and photoelectrochemistry of dye-incorporated clay-modified electrode
KAMAT P V
Univ. Notre Dame, radiation lab., Notre Dame IN 46556, USA
Journal: Journal of electroanalytical chemistry and interfacial electrochemistry, 1984, 163 (1-2) 389-394
ISSN: 0022-0728 Availability: CNRS-1150
No. of Refs.: 13 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: Switzerland
Language: English
Etudes dans le cas de **thionine** incorporee dans des couches minces d'argile fixees sur des electrodes de SnO SUB 2 et de Pt. Resultats obtenus par etudes de voltammetrie cyclique, ainsi que donnees sur la generation de l'effet photoelectrochimique sous l'effet d'un rayonnement de lambda >460 nm et en milieu aqueux contenant de l' **EDTA** 0,2 M

English Descriptors: Photoelectrochemistry; Semiconductor materials; Electrodes; Transition metal; Metal Oxides; Platinum; Thin layer electrode; Modified material; Chemical uptake; Thiazine dye; Electrochemical reaction; Cyclic voltammetry; Photoelectrochemical reaction; Aqueous solution; Organic solvent; Tin IV Oxides; Clay

French Descriptors: Photoelectrochimie; Semiconducteur; Electrode; Metal transition; Metal Oxyde; Platine-ACT; Electrode couche mince; Materiau modifie; Fixation chimique; Colorant thiazinique; Reaction electrochimique; Voltammetrie cyclique; Reaction photoelectrochimique; Solution aqueuse; Solvant organique; Etain IV Oxyde-ACT; Argile; **Thionine** ; Violet Lauth; **EDTA** -SUB

Classification Codes: 001C01H06

11/9/13 (Item 4 from file: 144)

04862032 PASCAL No.: 83-0108558
Effect of DNA and other polyanions on the EDTA **induced photoreduction of** thionine
MEDINI KANTA PAL; MANNA P C
Univ. Kalyani, dep. chemistry, Kalyani 741235, India
Journal: Makromol. Chem., 1982, 183 (11) 2811-2821
ISSN: 0025-116X Availability: CNRS-4111
No. of Refs.: 12 ref.

Document Type: P (Serial) ; A (Analytic)
Country of Publication: Switzerland
Language: English
Influence de l'ADN, de l'ARN et du polystyrenesulfonate

English Descriptors: DNA; Polyanion; Reduction; Photochemical reaction; Styrenesulfonate polymer; RNA; Basic dye

French Descriptors: DNA; Polyanion; Reduction; Reaction photochimique; Styrenesulfonate polymere; RNA; Colorant basique; **Thionine** -ENT; Tetracetique acide(**ethylenediamine**); Utilisation

Classification Codes: 780A02E

11/9/14 (Item 5 from file: 144)
DIALOG(R)File 144:Pascal

04003372 PASCAL No.: 75-0028838
MECHANISM OF PHOTOREDUCTION OF THIAZINE DYES BY EDTA **STUDIED BY FLASH PHOTOLYSIS. III. CONSEQUENCES OF A NEWLY FOUND PK SUB T OF** THIONINE **ON THE MECHANISM IN BASIC SOLUTIONS**
BONNEAU R; PEREYRE J
LAB. CHIM. PHYS. A, UNIV. BORDEAUX I, 33405 TALENCE,FRANCE
Journal: PHOTOCHEM. AND PHOTOBIOL., 1975, 21 (3) 173-177
Availability: CNRS-9410
No. of Refs.: 13 REF.
Document Type: P (SERIAL) ; A (ANALYTIC)
Country of Publication: UNITED KINGDOM
Language: ENGLISH
EQUILIBRE ENTRE LES FORMES NEUTRE ET MONOCATIONIQUE DE LA **THIONINE** TRIPLET, SUP 3 T ET SUP 3 TH SUP + (PK SUB T =8,9). PAS D'EQUILIBRE IDENTIQUE AVEC LE BLEU DE METHYLENE. DIFFERENCE DUE A LEUR PHOTOREDUCTION DIFFERENTE PAR **EDTA** . LE RENDEMENT QUANTIQUE DE FORMATION DU COLORANT SEMI REDUIT EST PROPORTIONNEL AU RENDEMENT DE REDUCTION GLOBAL. MECANISMES

English Descriptors: METHYLENE BLUE; ORGANIC DYE; MONOCYCLIC COMPOUND; ACIDITY CONSTANT; **EDTA** ; SULFUR NITROGEN HETEROCYCLE; REACTION MECHANISM ; FLASH PHOTOLYSIS; PHOTOCHEMICAL REACTION; REDUCTION; QUANTUM YIELD
English Generic Descriptors: PHYSICAL CHEMISTRY; PHOTOCHEMISTRY

French Descriptors: COLORANT ORGANIQUE; THIAZINE; BLEU METHYLENE; **THIONINE** ; HETEROCYCLE SOUFRE AZOTE; COMPOSE MONOCYCLIQUE; **EDTA** ; REDUCTION; REACTION PHOTOCHIMIQUE; CONSTANTE ACIDITE; RENDEMENT QUANTIQUE; PHOTOLYSE ECLAIR; MECANISME REACTION
French Generic Descriptors: CHIMIE PHYSIQUE; PHOTOCHIMIE

Classification Codes: 170A14B02A

11/9/22 (Item 13 from file: 144)
DIALOG(R)File 144:Pascal

00112095 PASCAL No.: 73-0032420
INDICADORES FOTO-REDOX EN AMPEROMETRA. DETERMINACIONES INDIRECTAS DE ANIONES CON ETILENODIAMINO TETRAACETO DIHYDROGENO DISODICO
(INDICATEURS PHOTO-REDOX EN AMPEROMETRIE. DOSAGE INDIRECT DES ANIONS AVEC L' EDTA **-H SUB 2 NA SUB 2)**
SIERRA F; SANCHEZ-PEDRENO C; PEREZ RUIZ T; MARTINEZ LOZANO C
C.S.I.C., DEP. QUIM. ANAL.,DE MURCIA
Journal: INFORM. QUIM. ANAL., 1973, 27 (2) 93-98
Availability: CNRS-2711
No. of Refs.: 6 REF.
Document Type: P (SERIAL)

Country of Publication: SPAIN
Language: SPANISH Summary Language: ENGLISH
EMPLOI DE **THIONINE** COMME INDICATEUR REDOX-PHOTOCHIMIQUE POUR LE TITRAGE INDIRECT DE CRO SUB 4 SUP 2- , SO SUB 4 SUP 2- , PO SUB 4 H SUB 2 SUP - ET (FE(CN) SUB 6) SUP 4-

English Descriptors: AMPEROMETRY; CHEMICAL ANALYSIS;CHEMICAL COMPOSITION; CHROMATES; ANIONIC COMPLEX; **EDTA** ; ANALYTICAL INDICATOR; REDOX INDICATOR ; PHOSPHATES; ABSORPTION SPECTROMETRY;ABSORPTION SPECTROSCOPY; SULFATES
English Generic Descriptors: ANALYTICAL CHEMISTRY
French Descriptors: **THIONINE** ; INDICATEUR ANALYTIQUE; CHROMATE; SULFATE; PHOSPHATE; FER II COMPOSE; FER II COMILEXE; CONPLEXE CYANO; COMPLEXE ANIONIQUE; ANALYSE CHIMIQUE; AMPEROMETRIE; TITRAGE AMPEROMETRIQUE; **EDTA** ; INDICATEUR REDOX; SPECTROMETRIE ABSORPTION; ANALYSE PAR; METHODE INDIRECTE; CYANO; ANALYSE
French Generic Descriptors: CHIMIE ANALYTIQUE

Classification Codes: 170C05B06

11/9/51 (Item 3 from file: 434)
DIALOG(R)File 434:SciSearch(R) Cited Ref Sci
(c) 1998 Inst for Sci Info. All rts. reserv.

01388440 Genuine Article#: CW889 Number of References: 20
Title: DETERMINATION OF IODIDE TRACES USING INHIBITORY EFFECT IN PHOTOCHEMICAL INTERACTION OF THIONINE **AND** ETHYLENEDIAMINETETRAACETIC **ACID**
Author(s): SIERRA F; SANCHEZPEDRENO C; PEREZRUIZ T; MARTINEZLOZANO C; HERNANDEZCORDOBA M
Corporate Source: FAC CIENCIAS MURCIA,CSIC,CTR COORDINADO,DEPT QUIM ANAL/MURCIA//SPAIN/
Journal: ANALES DE QUIMICA, 1977, V73, N1, P67-70
Language: SPANISH Document Type: ARTICLE
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11/3,KWIC/25 (Item 1 from file: 399)
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11/3,KWIC/30 (Item 6 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

106226561 CA: 106(26)226561c JOURNAL
Thionine blue as a photoredox indicator in titrations with EDTA. Determinations of metal ions, mixtures, and anions
AUTHOR(S): Perez Ruiz, T.; Martinez Lozano, C.; Tomas, V.; Yague, E.
LOCATION: Fac. Cienc. Quim. Mat., Univ. Murcia, Murcia, Spain,
JOURNAL: Quim. Anal. (Barcelona) DATE: 1986 VOLUME: 5 NUMBER: 2
PAGES: 164-79 CODEN: QUANEL ISSN: 0212-0569 LANGUAGE: Spanish

11/3,KWIC/31 (Item 7 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

101113892 CA: 101(14)113892q JOURNAL
Thionine and ferric chelate compounds as coupled mediators in microbial fuel cells
AUTHOR(S): Tanaka, Kazuko; Vega, Carmen A.; Tamamushi, Reita
LOCATION: Inorg. Chem. Lab., Inst. Phys. Chem. Res., Wako, Japan, 351
JOURNAL: Bioelectrochem. Bioenerg. DATE: 1983 VOLUME: 11 NUMBER: 4-6
PAGES: 289-97 CODEN: BEBEBP ISSN: 0302-4598 LANGUAGE: English

11/3,KWIC/32 (Item 8 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

99178976 CA: 99(22)178976e JOURNAL
Photochemical energy conversion by a thiazine photosynthetic-photoelectrochemical cell
AUTHOR(S): Pan, R. L.; Bhardwaj, R.; Gross, E. L.
LOCATION: Dep. Biochem., Ohio State Univ., Columbus, OH, 43210, USA
JOURNAL: J. Chem. Technol. Biotechnol., Chem. Technol. DATE: 1983
VOLUME: 33A NUMBER: 1 PAGES: 39-48 CODEN: JCTTDW ISSN: 0142-0356
LANGUAGE: English

11/3,KWIC/33 (Item 9 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

98071284 CA: 98(9)71284e JOURNAL
Effect of DNA and other polyanions on the EDTA induced photoreduction of thionine
AUTHOR(S): Pal, Medini Kanta; Manna, Pravash C.
LOCATION: Fac. Sci., Univ. Kalyani, Kalyani, 741235, India
JOURNAL: Makromol. Chem. DATE: 1982 VOLUME: 183 NUMBER: 11 PAGES: 2811-21 CODEN: MACEAK ISSN: 0025-116X LANGUAGE: English

11/3,KWIC/34 (Item 10 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

93060423 CA: 93(6)60423v JOURNAL
The use of thionine as a photoredox indicator in amperometric titrations of the mercury(I) ion with polyaminopolycarboxylated agents
AUTHOR(S): Sierra, F.; Cebrian, A.; Hidalgo de Cisneros, J. L. H.
LOCATION: Dep. Quim. Anal., Fac. Cienc. Murcia, Murcia, Spain,
JOURNAL: Afinidad DATE: 1979 VOLUME: 36 NUMBER: 364 PAGES: 515-17
CODEN: AFINAE ISSN: 0001-9704 LANGUAGE: Spanish

11/3,KWIC/35 (Item 11 from file: 399)

DIALOG(R)File 399:CA SEARCH(R)

88145569 CA: 88(20)145569u JOURNAL
Determination of periodate with photoreduced thionine
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Perez Ruiz, T.; Martinez Lozano, C.
LOCATION: Dep. Anal. Chem., Univ. Murcia, Murcia, Spain
JOURNAL: Anal. Chim. Acta DATE: 1977 VOLUME: 94 NUMBER: 1 PAGES: 129-33 CODEN: ACACAM ISSN: 0003-2670 LANGUAGE: English

11/3,KWIC/36 (Item 12 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

88083095 CA: 88(12)83095a JOURNAL
Determination of trace iodide by using its inhibiting effect on the photochemical reaction between thionine and EDTA
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Perez Ruiz, T.; Martinez Lozano, C.; Hernandez Cordoba, M.
LOCATION: Dep. Quim. Anal., CSIC, Murcia, Spain
JOURNAL: An. Quim. DATE: 1977 VOLUME: 73 NUMBER: 1 PAGES: 67-70
CODEN: ANQUBU LANGUAGE: Spanish

11/3,KWIC/37 (Item 13 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

86162741 CA: 86(22)162741v JOURNAL
Photoinduced potentials by photoredox systems and the effects of the addition of EDTA
AUTHOR(S): Kaneko, Masao; Yamada, Akira
LOCATION: Inst. Phys. Chem. Res., Wako, Japan
JOURNAL: Rikagaku Kenkyusho Hokoku DATE: 1976 VOLUME: 52 NUMBER: 6
PAGES: 210-15 CODEN: RKKHAO LANGUAGE: Japanese

11/3,KWIC/38 (Item 14 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

85195209 CA: 85(26)195209k TECHNICAL REPORT
Evaluation of some thionine redox systems as potential regenerative photogalvanic batteries
AUTHOR(S): Fine, Dwight A.; Fletcher, Aaron N.
LOCATION: Nav. Weapons Cent., China Lake, Calif.
JOURNAL: U. S. NTIS, AD Rep. DATE: 1976 NUMBER: AD-A021424 PAGES: 25 pp. CODEN: XADRCH LANGUAGE: English CITATION: Gov. Rep. Announce. Index (U. S.) 1976, 76(9), 121 AVAIL: NTIS

11/3,KWIC/39 (Item 15 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

82154984 CA: 82(23)154984d JOURNAL
Photochemical determination of a new acid-base equilibrium of thionine in its triplet state. Application to the photoreactivity of thiazine dyes in aqueous solution
AUTHOR(S): Bonneau, Roland; Pereyre, Josette; Joussot-Dubien, Jacques
LOCATION: Lab. Chim. Phys. A, Univ. Bordeaux I 351, Talance, Fr.
JOURNAL: Mol. Photochem. DATE: 1974 VOLUME: 6 NUMBER: 3 PAGES: 245-52
CODEN: MLPCBL LANGUAGE: English

DIALOG(R)File 399:CA SEARCH(R)

74008313 CA: 74(2)8313c JOURNAL
Photochemical reduction of thionine and other thiazine dyes by Co(II)EDTA complex in a heterogeneous system
AUTHOR(S): Singhal, G. S.; Rabinowitch, Eugene
LOCATION: Chem. Dep., State Univ. New York, Albany, N. Y.
JOURNAL: J. Chem. Phys. DATE: 1970 VOLUME: 53 NUMBER: 10 PAGES: 4109-10 CODEN: JCPSA6 LANGUAGE: English

11/3,KWIC/46 (Item 22 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

73051836 CA: 73(10)51836s JOURNAL
Formation and stabilization of high valence states in silver
AUTHOR(S): Sanchez=Manzanares, Jose A.
JOURNAL: An. Univ. Murcia, Cienc. DATE: 1968 VOLUME: 26 NUMBER: 1-4 PAGES: 57-161 CODEN: AUMCB5 LANGUAGE: Spanish

11/3,KWIC/47 (Item 23 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

72122159 CA: 72(24)122159r JOURNAL
Photopolymerization using methylene blue-reducing agent system and its application to photoelectrochemical cell
AUTHOR(S): Kamiya, Nobuyuki; Okawara, Makoto
LOCATION: Res. Lab. Resour. Util., Tokyo Inst. Technol., Tokyo, Japan
JOURNAL: Kogyo Kagaku Zasshi DATE: 1969 VOLUME: 72 NUMBER: 12 PAGES: 2639-44 CODEN: KGKZA7 LANGUAGE: Japanese

11/3,KWIC/48 (Item 24 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

72084849 CA: 72(16)84849f JOURNAL
Photochemical reduction of thionine by cobalt(II) EDTA complex in water-ether emulsion
AUTHOR(S): Srinivasan, V.; Rabinowitch, E.
LOCATION: Dep. of Bot., Univ. of Illinois, Urbana, Ill.
JOURNAL: J. Chem. Phys. DATE: 1970 VOLUME: 52 NUMBER: 3 PAGES: 1165-8
CODEN: JCPSA6 LANGUAGE: English
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(54) BIOCIDAL PROTEIN

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(57) **ABSTRACT**

The invention relates to novel nucleic acid and protein sequences from the mung bean *Vigna radiata*. The nucleic acid sequence, isolated from a bruchid resistant mung bean line, encodes a thionin-like protein with insecticidal properties.

BIOCIDAL PROTEIN

RELATED APPLICATIONS

[0001] This application is a divisional application and claims priority to U.S. application Ser. No. 09/686,332, filed Oct. 11, 2000, the contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] Advances in biotechnology have enabled the generation of plants which express recombinant proteins. Thus, plants can be engineered to overproduce a variety of polypeptides with desirable qualities. Such polypeptide can include enzymes which produce secondary metabolites, proteins with medicinal or pharmaceutical properties, and proteins which endow the plants with new traits, for example, resistance to diseases, pathogens, and environmental conditions.

[0003] Given the vulnerability of agricultural crops to damage by insects, and other pests and pathogens, the ability to provide additional protective means and agents is of considerable importance. Moreover, traditional breeding techniques have identified plant lines with Mendelian traits endowing resistance to pests and pathogens. Modern molecular biological techniques can now be applied to isolate the critical nucleic acids and proteins with these properties in order to enhance the resistance of more sensitive plants or to antagonize the pests and pathogens in a variety of scenarios.

SUMMARY OF THE INVENTION

[0004] The invention is based, in part, on the discovery of a novel mung bean nucleic acid which is expressed in a mung bean plant line that is resistant to insect attack, but not expressed in sensitive plant lines. The nucleic acid encodes a polypeptide which has insecticidal activity and which has similarity to thionin proteins. The sequence of the mung bean thionin nucleic acid (SEQ ID NO: 1), designated as "VrCRP", is shown below:

5'-ACCTCAACAATTCATCACTCATGGAGAGAAAAACTTTCAGCTTCTTG

TTCTCGCTCCTTCTCGTCTTAGCCTCTGATGTGGCCGTAGAGAGAGGAGA

GGCTAGAACTTGTATGATAAAGAAAGAAGGGTGGGGAAAATGCTTAATTG

ACACCACCTGTGCACATTCGTGCAAGAACCGCGGTTACATAGGTGGAGAT

TGCAAAGGCATGACGCGCACCTGCTATTGCCTCGTCAACTGTTGAACCCT

TTTCGAATATCATATCATCTTATCACAAATAAATATAGCAGCATCACTGC

TACTAGTACCGCCCTCCGCACCACGCCCT-3'

[0005] The initiator and terminator codons are underlined and in boldface. The sequence of the mung bean thionin polypeptide sequence (SEQ ID NO:2), designated as "VrCRP", is shown below:

MERKTFSFLFSLLLVLASDVAVERGEARTCMIKKEGWGKCLIDTTCAHSC
KNRGYIGGDCKGMTRTCYCLVNC

[0006] The invention is also based on the discovery the a polypeptide derived from VrCRP which has the VrCRP signal sequence removed is biologically active as an insecticide and fungicide. This polypeptide is encoded by the nucleic acid sequence (SEQ ID NO:3) below:

GAGAGAGGAGAGGCTAGAACTTGTATGATAAAGAAAGAAGGGTGGGGAAA

ATGCTTAATTGACACCACCTGTGCACATTCGTGCAAGAACCGCGGTTACA

TAGGTGGAGATTGCAAAGGCATGACGCGCACCTGCTATTGCCTCGTCAAC

TGTTGA

[0007] The polypeptide sequence (SEQ ID NO:4) of this form of VrCRP lacking the signal sequence is shown below:

ERGEARTCMIKKEGWGKCLIDTTCAHSCKNRGYIGGDCKGMTRTCYCLV
NC

[0008] Accordingly, in one aspect, the invention features isolated nucleic acid sequences which include a nucleic acid sequence comprising the nucleotide sequence of SEQ ID NO: 1. The nucleic acids of the invention can further include nucleic acids which hybridize under stringent conditions to SEQ ID NO:1, as well as nucleic acids which are at least 50% identical, e.g., at least 60%, 70%, 80%, 90%, or 95% identical, to SEQ ID NO: 1. Such nucleic acid sequences can encode a polypeptide which inhibits translation of messenger RNAs in a wheat germ extract, a polypeptide which has insecticidal activity, e.g., insecticidal activity against bruchids such as *Callosobruchus chinensis, Callosobruchus maculates*, and *Zabrotes subfasciatu*, or a polypeptide which has anti-fuingal activity, e.g., against *Rhizoctonia solani.*

[0009] In another aspect, the invention features polypeptides comprising the amino acid sequence of SEQ ID NO:2. Featured polypeptides also include polypeptides which are at least 50% identical, e.g., at least 60%, 70%, 80%, 90%, or 95% identical, to SEQ ID NO:2. Such polypeptides can have at least one, two, three, four, five, eight, ten, twelve, or twenty conservative amino acids substitutions. The polypeptides can inhibit translation of messenger RNAs in a wheat germ extract, can have insecticidal activity, e.g., insecticidal activity against bruchids such as *Callosobruchus chinensis, Callosobruchus maculates*, and *Zabrotes subfasciatus*, or can have anti-fungal activity, e.g., against *Rhizoctonia solani.* Also encompassed by the invention are nucleic acid sequences encoding such polypeptides.

[0010] The featured polypeptides can be recombinant and/or purified. For example, they can be overexpressed in a variety of host cells, such as *E. coli*, Sf9 insect cells, plant cells and mammalian tissue culture cells using overexpression vectors known in the art. Lysates are made from the host cells, e.g., after overexpression is induced if induction is required. The polypeptides are purified from the lysate. Alternatively, the polypeptides are secreted, by the inclusion nucleic acid sequences encoding the signal peptide or a heterologous signal peptide. In another example, the featured polypeptides are encoded by a transgene and overproduced in a plant or a plant tissue. The plant is harvested and the polypeptides purified from the plant.

[0011] The purified and/or recombinant polypeptides can be formulated a composition. The composition can include an agriculturally acceptable carrier, e.g., one described

below. The composition can contain the polypeptide at a concentration of about 0.005% to 10%, or about 0.01% to 1%, or about 0.1% to 0.5% by weight of composition. The composition can further include a cyclopeptide alkaloid, e.g., vignatic acid A and vignatic acid B, e.g., a cyclopeptide alkaloid with insecticidal properties. The composition can also include other desirable compounds, e.g., protease inhibitors, endotoxins, and the like. The contemplated compositions can have insecticidal activity, e.g., against bruchids such as *Callosobruchus chinensis, Callosobruchus maculates*, and *Zabrotes subfasciatu*, and/or anti-fungal activity, e.g., against *Rhizoctonia solani*. The compositions can be applied to plants and their environs by methods described below.

[0012] The nucleic acids of the invention can also include a heterologous promoter such that the promoter is operably linked to a coding genomic nucleic acid or a cDNA. The promoter can direct transcription of the nucleic acid in wounded or pathogen infected cells. The promoter can be induced by a signalling molecule, e.g., methyl jasmonate, salicylic acid, ethylene, absiscic acid, gibberillins, $HgCl_2$, and H_2O_2. The invention also features transformed cells which contain such nucleic acids, i.e., an aforementioned nucleic acid operably linked to a heterologous promoter. Also included are transgenic plants whose genomic DNA includes such nucleic acids, as are transgenic seeds from such plants.

[0013] A "purified polypeptide", as used herein, refers to a polypeptide that has been separated from other proteins, lipids, and nucleic acids with which it is naturally associated. The polypeptide can constitutes at least 10, 20, 50 70, 80 or 95% by dry weight of the purified preparation.

[0014] An "isolated nucleic acid" is a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid, or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. The term therefore covers, for example, (a) a DNA which has the sequence of part of a naturally occurring genomic DNA molecule but is not flanked by both of the coding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Specifically excluded from this definition are nucleic acids present in mixtures of different (i) DNA molecules, (ii) transfected cells, or (iii) cell clones: e.g., as these occur in a DNA library such as a cDNA or genomic DNA library.

[0015] The term "substantially pure" as used herein in reference to a given polypeptide means that the polypeptide is substantially free from other biological macromolecules. The substantially pure polypeptide is at least 75% (e.g., at least 80, 85, 95, or 99%) pure by dry weight. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

[0016] The "percent identity" of two amino acid sequences or of two nucleic acids is determined using the algorithm of Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 87:2264-68, 1990, modified as in Karlin and Altschul Proc. Natl. Acad. Sci. USA 90:5873-77, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. *J. Mol. Biol.* 215:403-10, 1990. BLAST nucleotide searches can be performed with the NBLAST program, score=100, wordlength-12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of the invention. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul et al., *Nucleic Acids Res.* 25(17):3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

[0017] As used herein, the term "transgene" means a nucleic acid sequence (encoding, e.g., one or more subject thionin polypeptides), which is partly or entirely heterologous, i.e., foreign, to the transgenic plant or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic plant or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the plant's genome in Such a way as to alter the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout). A transgene can include one or more transcriptional regulatory sequences and any other nucleic acid, such as introns, that may be necessary for optimal expression of the selected nucleic acid, all operably linked to the selected nucleic acid, and may include an enhancer sequence.

[0018] As used herein, the term "transgenic cell" refers to a cell containing a transgene. As used herein, a "transgenic plant" is any plant in which one or more, or all, of the cells of the plant includes a transgene. The transgene can be introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by T-DNA mediated transfer, electroporation, or protoplast transformation. The transgene may be integrated within a chromosome, or it may be extrachromosomally replicating DNA.

[0019] As used herein, the term "tissue-specific promoter" means a DNA sequence that serves as a promoter, i.e., regulates expression of a selected DNA sequence operably linked to the promoter, and which effects expression of the selected DNA sequence in specific cells of a tissue, such as a leaf, a root, or a stem.

[0020] As used herein, the term "hybridizes under stringent conditions" refers to conditions for hybridization in 6× sodium chloride/sodium citrate (SSC) at about 45° C., followed by one or more washes in 0.2× SSC, 0.1% SDS at 50-65° C.

[0021] A "heterologous promoter", when operably linked to a nucleic acid sequence, refers to a promoter which is not naturally associated with the nucleic acid sequence.

[0022] As used herein, an agent with "insecticidal activity" is an agent which when tested by the following assay

has measurable insect-killing activity. In the assay, the agent is combined with mung bean flour produced from an insect-sensitive mung bean strain and packed as an artificial bean. Insect eggs are placed on the artificial bean. The timing and number of hatched insects are measured. An agent with "insecticidal activity" demonstrates delayed hatched, e.g., a delay of about 2, 4, 5, 7, 10, or 14 days. Alternatively, the agent can prevent hatching, e.g., only about 80%, 60%, 40%, 30%, 10%, or 0% of the eggs hatch after 14 days.

[0023] As used herein, an agent with "fungicidal activity" is an agent which when tested in the following assay produces a measurable zone of growth inhibition. The agent, in an acceptable solvent, is soaked in a sterile filter disc which is placed on an agar plate top spread with a fungus, e.g., *Rhizoctonia solani*. The plates are incubated, e.g., for 36-48 hours, and then zone of growth inhibition around each filter disc is measured. An agent with "anti-fungal activity" produces a zone of inhibition of about 1, 2, 3, 4, 5 mm or greater after 36 hours of fungus growth.

[0024] As used herein, an agent which "inhibits messenger RNA translation in a wheat germ extract" is an agent which when present in an in vitro translation reaction obtained by combining a messenger RNA with a wheat germ extract, e.g., a commercial wheat germ extract, prevents incorporation of [35S] methlionine into an acid insoluble fraction by at least 30%, e.g., at least about, 50%, 75%, or 100%

[0025] The discovery of a polypeptide thionin from insect resistant mung beans with insecticidal properties and its encoding nucleic acid sequence has a variety of commercial and agriculture benefits. The polypeptide can be used in a composition, e.g., a composition which includes agriculturally acceptable carriers, or other agents, to protect plants from insects, and other pests and pathogens. The composition can be formulated and applied by methods described herein in order to protect a plant. In another aspect, the nucleic acid can be used to generate a transgenic plant which expresses the thionin to thereby protect the plant. By conferring resistance to damage by insects, these strategies are of considerable economic benefit.

[0026] Other features, objects, and advantages of the invention will be apparent from the description and from the claims.

DETAILED DESCRIPTION

[0027] The invention provides nucleic acids and polypeptides with insecticidal properties. These molecules can be isolated from a bruchid resistant line of the mung bean *Vigna radiata*. Moreover, isolated nucleic acids and isolated polypeptides of the invention can be used to provide pest and pathogen resistance in a variety of scenarios.

[0028] Assaying Resistance

[0029] A simple bioassay is utilized in order to assess the insecticidal properties of the plant lines, nucleic acids, and polypeptides featured herein. Bruchids, (e.g., bruchids obtained from the Asian Vegetable Research and Development Center (AVRDC), P.O. Box 42, Shanhua, Tainan 741, Taiwan, are maintained on sensitive mung bean seeds, e.g., on VC1973A seeds. To assay mung bean plants for resistance, seeds are obtained from the plant in question. Six bruchid eggs are placed on a seed. Multiple seeds from a single plant in question can be so tested. The seeds are incubated at 25° C. and 60% humidity, and observed daily. The number of live bruchids emerging each day is monitored, and compared to data obtained from control sensitive and resistant lines.

[0030] In order to determine if a composition, e.g., a formulation containing a polypeptide featured in this invention, has insecticidal properties, the above bioassay is easily adapted to testing the composition. Flour is produced from sensitive mung beau seeds, combined with the composition and molded into an artificial seed following the method of Shade et al.(1986) *Bio/Technology* 12:793-796. Again, six bruchid eggs are placed on each seed. The artificial seeds are monitored as the real seeds described above.

[0031] Polypeptide Expression

[0032] The nucleic acids featured herein can be utilized to express polypeptides with insecticidal properties. Methods for expressing and obtaining polypeptides from coding nucleic acids sequences are routine in the art. The coding nucleic acid sequence for mung bean thionin can be cloned into an expression vector, for example, a bacterial expression vector. The vector can have an inducible promoter, e.g., the lac promoter or a derivative thereof. Alternatively, the vector can have a T7 polymerase promoter. The vector can also include nucleic acid sequences encoding a polypeptide tag or fusion gene to facilitate purification of an inserted heterologous coding sequence. For example, the tag can be a short peptide epitope for an antibody, or a purification handle such as hexa-histidinie. The tag can encode a completely folded polypeptide, such as glutathiolle-S-transferase, maltose binding protein, or chitin binding domain. Between the tag and the inserted coding sequence can be sequence encoding a site specific protease recognition site. The vector can include a sequence to export the desired polypeptide into the periplasm.

[0033] The vector is transformed into a host cell, e.g., *E. coli* DH5α. Transformed cells can be propagated, and treated with an inducer to activate polypeptide expression.

[0034] If the expressed polypeptide is fused to a tag or fusion gene with a purification handle, the polypeptide can be easily purified from a clarified cell lysate with an appropriate affinity column, e.g., Ni^{2+} NTA resin for bexa-histidine, glutathione agarose for GST, amylose resin for maltose binding protein, chitin resin for chitin binding domain, and antibody affinity columns for epitope tagged proteins. Tile desired polypeptide can be eluted form the affinity column, or if appropriate cleaved from the column with a site specific protease. If the protein is not tagged for purification, routine methods in the art can be used to develop procedures to isolate it from cell lysates, periplasm, or the media (see, e.g., Scopes, RK (1994) *Protein Purification: Principles and Practice*, 3rd ed., New York: Springer-Verlag).

[0035] Analogs of mung bean thionin include thionins (or biologically active fragments thereof) whose sequences differ from the wild-type sequence by one or more conservative amino acid substitutions or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not abolish bruchid killing activity. The following table list suitable amino acid substitutions:

TABLE 1

CONSERVATIVE AMINO ACID REPLACEMENTS

For Amino Acid	Code	Replace with any of
Alanine	A	Gly, Ala, Cys
Arginine	R	Lys, Met, Ile,
Asparagine	N	Asp, Glu, Gln,
Aspartic Acid	D	Asn, Glu, Gln
Cysteine	C	Met, Thr
Glutamine	Q	Asn, Glu, Asp
Glutamic Acid	E	Asp, Asn, Gln
Glycine	G	Ala, Pro,
Isoleucine	I	Val, Leu, Met
Leucine	L	Val, Leu, Met
Lysine	K	Arg, Met, Ile
Methionine	M	Ile, Leu, Val
Phenylalanine	F	Tyr, His, Trp
Proline	P	
Serine	S	Thr, Met, Cys
Threonine	T	Ser, Met, Val
Tyrosine	Y	Phe, His
Valine	V	Leu, Ile, Met

[0036] Promoters

[0037] For polypeptides which confer resistance to pests or pathogens, the isolated coding nucleic acid sequence can be operably linked to a heterologous promoter. The promoter can activate transcription and, thus, polypeptide expression in a subset of tissues. Alternatively, the promoter can alter transcription rates in response to environmental stimuli, systemic signals, or intracellular signals. For example, promoters are known which respond to excessive heat, tissue injury, pathogen infection, or cell wounding, e.g., wounding due to pest attack. Known signals for promoters included methyl jasmoonate, absiscic acid, gibberillins, salicylic acid, ethylene, $HgCl_2$, and H_2O_2. Methyl jasmonate responsive promoters include vspB (Mason et al. (1993) *Plant Cell* 5:241-251), and the tomato HMG2 promoter (U.S. Pat. No. 5,689,056). An example of a gibberillin response promoter is the Amy1/6-4 promoter of rice (Skriver et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:7266-7270). Promoters which respond to pathogen infection include the grape stilbene synthase promoter (U.S. Pat. No. 6,072,103). Promoters which respond to cell wounding include the win1 and win2 promoters (Weiss and Bevan (1991) *Plant Physiol.* 96:943-951), and the PinII promoter (U.S. Pat. No. 5,684,239). For example, any of these promoters can be operably linked to a nucleic acid sequence of SEQ ID NO: 1 in order to regulate expression of the polypeptide of SEQ ID NO:2. Similarly, any of these promoters can be operably linked to variants or fragments of SEQ ID NO: 1, or other similar coding nucleic acid sequences.

[0038] Methods of Transforming Plant Cells

[0039] A nucleic acid construct of the present invention can be transformed into a plant cell to produce a desired transgenic plant or plant cell. Methods for transforming plant cells with nucleic acid are routine in the art. Further, the plant cells can be transformed with multiple constructs, e.g., sequentially or concurrently. Depending on the desired physiological and agronomic properties of a plant species, and the nucleic acid construct of the present invention, a target plant or plant cell for transformation can include a species from maize, wheat, rice, soybean, tomato, tobacco, carrots, peanut, potato, sugar beets, sunflower, yam, Arabidopsis, rape seed, sunflower, and petunia.

[0040] One implementation of the current invention utilizes Agrobacterium, to introduce the desired construct into plant cells such as in U.S. Pat. Nos. 5,177,010, 5,104,310, 5,149,645, 5,469,976, 5,464,763, 4,940,838, 4,693,976, 5,591,616, 5,231,019, 5,463,174, 4,762,785, 5,004,863, and 5,159,135; and European Patent Applications 1 6718, 290799, 320500, 604662, 627752, 0267159, and 0292435). The method can be used with both dicotyledonous plants cells (Bevan et al. (1982) *Ann. Rev. Genet.* 16:357-384; Rogers et al. (1986) *Methods Enzymol.* 118:627-641), and monocotyledonous plant cells. (Hernalsteen et al. (1984) *EMBO J* 3:3039-3041; Hooykass-Van Slogteren et al. (1984) *Nature* 311:763-764; Grimsley et al. (1987) *Nature* 325:1677-179; Boulton et al. (1989) *Plant Mol. Biol.* 12:31-40.; Gould et al. (1991) *Plant Physiol.* 95:426-434). The method employs binary Agrobacterium T-DNA vectors (Hoekema et al. (1983) *Nature* 03:179; Bevan, 1984, *Nuc. Acid Res.* 12:8711-8721), and the co-cultivation procedure (Horsch et al., 1985, *Science* 227:1229-1231).

[0041] Additional steps may be required to prepare a desired nucleic acid sequence for plant transformation. For example, in order to utilize T-DNA mediated transformation, the thionin coding sequence, operably linked to a heterologotus promoter, is ligated into a binary vector, between the left and right border sequences of T-DNA. The binary vector further includes an Hph gene coding for hygromycin resistance. The binary vector containing the desired construction is transformed into an *E. coli* strain, e.g., DH5α. Subsequently, the binary plasmid is transferred into an Agrobacterium, e.g., Agrobacterium strain LBA4404, using a triparental mating.

[0042] Meanwhile, plants are prepared to receive the T-DNA with the transgene. Leaf discs are obtained from axenically grown tobacco seedlings. The discs are incubated for 8 hours on sterile filter papers overlaid on tobacco nurses cells on a feeder plate containing modified MS medium with Nitsch vitamins, 100 ml/L myo-inositol, 30 mg/L sucrose, 0.4 mg/L BAP, 1 mg/L 2,4-D (dichliorophenioxyacetic acid), 8 ml/L agar. To establish co-cultivation, the filters bearing the leaf disks are submersed in a suspension of the Agrobacterium bearing the desired binary vector, the bacteria being a concentration of approximately $1 \cdot 10^9$ cell/ml, and vacuum infiltrated (3×1 minute). The filters and leaf discs are incubated on the nurse plate for 48 hours at 25° C. with indirect light. Then the discs are transferred to selection/regeneration plates containing MS salts, Nitsch vitamins, 100 ml/L myo-inositol, 20 g/L sucrose, 2 mg/L zeatin, 4 g/L agar, 500 μg/ml carbemicillin and an appropriate antibiotic, e.g., G418 to select for the hygromllycill resistance gene. The plates are placed in a growth chamber at 25° C. for 18 hours with light. The resulting shoots were transferred to rooting media, grown into plantlets, transferred to soil, and grown into plants in a green hose. One skilled in the art can adapt this method to transform other species of plants.

[0043] Other methods for transforming plant cells are available. Of particular utility for transforming monocotyledonous plants or plant cells are methods of protoplast transformation which include, but are not limited to, protoplast transformation through calcium-, polyethylene glycol (PEG)- or electroporation-mediated uptake of naked DNA (see Paszkowski et al., 1984, *EMBO J*3:2717-2722, Potrykus et al. 1985, *Molec. Gen. Genet.* 199:169-177; Fromm

et al., 1985, *Proc. Nat. Acad. Sci. USA* 82:5824-5828; Shimamoto, 1989, *Nature* 338:274-276), microinjection, silicon carbide mediated DNA uptake (Kaeppler et al., 1990, *Plant Cell Reporter* 9:415-418), and microprojectile bombardment (see Klein et al., 1988, *Proc. Nat. Acad. Sci. USA* 85:4305-4309; Gordon-Kamm et al., 1990, *Plant Cell* 2:603-618), whiskers technology (see U.S. Pat. Nos. 5,302,523 and 5,464,765), and viral vector systems (see, U.S. Pat. Nos. 5,316,931, 5,589,367, 5,811,653, and 5,866,785).

[0044] A transformed plant or transformed plant tissue can be assayed for resistance to pathogens, insects, and other pests (e.g., by a field trial or by a method described herein).

[0045] Agricultural Compositions

[0046] Polypeptides, e.g., mung bean thionin protein, can be formulated as a composition which is applied to plants in order to confer insect, pest, or pathogen resistance. The composition can be prepared in a solution, e.g., an aqueous solution, at a concentration from about 0.005% to 10%, or about 0.01% to 1%, or about 0.1% to 0.5% by weight of polypeptide content. The solution can comprise an organic solvent, e.g., glycerol or ethanol. Alternatively, the composition can be formulated with one or more agriculturally acceptable carriers. Agricultural carriers can include: clay, talc, bentonite, diatomaceous earth, kaolin, silica, benzene, xylene, toluene, kerosene, N-methylpyrrolidone, alcohols (methanol, ethanol, isopropanol, n-butanol, ethylene glycol, propylene glycol, and the like), and ketones (acetone, methylethyl ketone, cyclohexanone, and the like). The formulation can optionally further include a stabilizer, spreading agent, wetting extenders, dispersing agents, sticking agents, disintegrators, and other additives, and can be prepared as a liquid, a water-soluble solid (e.g., tablet, powder or granule), or a paste.

[0047] Prior to application, the solution can be combined with another desired composition such a insecticide, germicide, fertilizer, plant growth regulator and the like. The solution may be applied to the plant tissue, for example, by spraying, e.g., with an atomizer, by drenching, by pasting, or by manual application, e.g., with a sponge. The solution can also be distributed from an airborne source, e.g., an aircraft or other aerial object, e.g., a fixture mounted with an apparatus for spraying the solution, the fixture being of sufficient height to distribute the solution to the desired plant tissues. Alternatively, the composition can be applied to plant tissue from a volatile or airborne source. The source is placed in the vicinity of the plant tissue and the composition is dispersed by diffusion through the atmosphere. The source and the plant tissue to be contacted can be enclosed in an incubator, growth chamber, or greenhouse, or can be in sufficient proximity that they can outdoors.

[0048] In another implementation, if the composition is distributed systemically thorough the plant, the composition can be applied to tissues other than the leaves, e.g., to the stems or roots. Thus, the composition can be distributed by irrigation. The composition can also be injected directly into roots or stems.

[0049] Without further elaboration, it is believed that the above description has adequately enabled the present invention. The following examples are, therefore to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All of the publications cited herein are hereby incorporated by reference in their entirety.

[0050] Materials and Methods

[0051] Plant and Insect Materials

[0052] Two nearly isogenic lines of mung bean *Vigna radiata* L. VC 1973A and VC6089A were obtained from the Asian Vegetable Research and Development Center (AVRDC). VC6089A (BC6S2) populations were crossed to wild mung bean *Vigna radiata TC*1966 as donor parent and breeding line VC1973A as recurrent parent. Azuki bean weevil *Callosobruchus chinensis* (L.) was also obtained from AVRDC that has been maintained on VC1973A at 25° C. in a growth chamber.

[0053] Isolations and Analyses of RNA and DNA

[0054] Total RNA was isolated via a modified hot phenol extraction method (Verwoerd et al. (1989) *Nucl Acids Res* 17:2362). Polyadenylated RNA was prepared by Oligo(dT)-cellulose affinity column (Maniatis et al. (1982) *Molecular Cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). RNA concentration was determined by measuring its absorbance at* 260 nm and verified by agarose gel electrophoresis and ethidium bromide staining. To confirm equal loading of total RNA on RNA gel blots, a 0.2 kb fragment of VrCRP was PCR amplified and used as a probe. Genomic DNA isolation was performed via a modified CTAB extraction method (Dellaporta et al. (1983) *Plant Mo. Biol. Rep.* 1:19). DNA gel blot analysis, including restriction, fragment length polymorphism, and downward alkaline capillary transfer to a positively charged nylon membrane (Hybond N+, Amersham Pharmacia Biotech, Baue D'Urfe, Canada) were performed as described by Young et al. ((1992) *Theor. Appl. Genet.* 84:839-844) and Chomczynski ((1992) *Anal. Biochem.* 201:134-139). Hybridization of the membrane was performed under high-stringency condition at 65° C. overnight, and after hybridization the membrane was washed twice with 2xSSC/1% SDS for 15 min at room temperature, and then washed twice with 0.2xSSC/1% SDS at 55° C. for 15 min (1xSSC is 0.15M NaCl, 0.015 M sodium citrate, pH 7.0). RNA gel blot analyses were also carried out as described by Chomczynski (1992) supra. RNAs were downward alkaline capillary-transferred onto Hybond N+ nylon membranes and cross-linked with a Stratalinker™ UV Crosslinker 1800. Hybridization and washing of the membranes was performed as described previously. Probes for both DNA and RNA gel blot analysis were synthesized from random-labeled isolated DNA inserts with α-^{32}P-dCTP. The membranes were exposed to phosphorimager, and scanned.

[0055] Suppression Subtractive Hybridization

[0056] Suppression subtractive hybridization (SSH) was performed on VC6089A cDNA and matched VC1973A cDNA, using the PCR select cDNA subtraction kit (Clontech Laboratories Inc., CA) according to the manufacturer's recommendations. VC6089A and VC1973A seeds were collected 15 DAF (days after flowering). mRNA was prepared from the seeds as described above, and reverse transcribed to generate cDNA, which is then converted to double-stranded DNA. The two cDNA populations were subtracted as described (Akopyants et al., ((1998) *Proc. Natl. Acad. Sci. USA* 95: 13108-13113). Briefly, the VC6089A cDNA, designated the "tester DNA", was digested with a restriction enzyme, e.g., AluI, and divided into two pools. Each pool was ligated to a different linker adaptor. The VC1973A DNA

was used as the "driver DNA," and was restricted with the same enzyme. Each pool of VC6089A cDNA was denatured and hybridized to an excess of digested driver DNA. Subsequently, the mixtures were cooled, and the DNA ends were extended in order to incorporate the adaptor end into the driver DNA strand. Finally, the two pools were combined, denatured, and reannealed. The ends of the reannealed strands were filled in with DNA polymerase. PCR amplification was used to amplify fragments that had two different linkers at each end, one from each pool. The procedure highly favors amplification of DNAs which were unable to anneal to DNA in the driver DNA population, i.e., cDNAs were are more abundant in VC6089A seeds than VC1973A seeds.

[0057] The amplified DNAs were cloned into the pGEM-T vector, transformed into competent *E. coli* cells, and grown in 96-well dishes. Each clone was transferred to a nitrocellulose membrane for blot hybridization to identify vectors with inserts. Inserts were amplified by PCR using the universal primers T7 and SP6, sequenced, and used for Northern blot analysis against mRNA from sensitive (VC1973A) and resistant (VC6089A) seeds. One DNA fragments which was differentially expressed in VC6089A contains nucleic acid encoding VrCRP.

[0058] Sequencing of DNAs and Peptides

[0059] DNA sequencing was carried out using a ABI autosequencer 377. N-terminal amino acid sequences were determined by automatic Edman degradation method using a gas-phase protein sequencer (Applied Biosystems, Model 473A). After SDS-PAGE separation, polypeptides were electroblotted onto a PVDF membrane and detected by Coomassie blue staining. Bands corresponding to the desired polypeptides were cut and processed for amino acid sequencing.

[0060] Construction of an Expression Vector

[0061] Two degenerate oligonucleotides were synthesized: VrCRF (5'-CAT GCC ATG GAG AGA GGA GAG GCT AGA AC-3') and VrCRR (5'-TCC CCC GGG ACA GTT GAC GAG GCA ATA-3'). VrCRF corresponds to the degenerate codons of the amino acids Glu^{23} to Arg^{28} (N-terminal amino acid methionine of the signal peptide is numbered as amino acid 1) of VrCRP and has a sense orientation and a NcoI site. VrCRR corresponds to the degenerate codons of the amino acids Tyr^{68} to Cys^{73} of VrCRP and has an antisense orientation and a SmaI site. PCR was performed essentially by the method of DNA polymerase chain reaction (Saiki et al., 1988), using 5 ng of VrCRP as target DNA, 10 pmol each of VrCRR and VrCRF, 250 nmol each of the dNTPs and 2.5 U Taq polymerase (Promega) in a total volume of 100 μL. The amplification program included an initial step at 95° C. for 5 min, 25 cycles (95° C. for 1 min, 55° C. for 1 min, and 72° C. for 1 min) and a final step at 72° C. for 10 min. The 171 bp amplification product was purified on a 1% agarose (FMC BioProducts) gel and excised from the gel with a razor blade (Chuang et al. (1994) *Biotechniques* 17:634-636). The sequence of the amplification product was confirmed by DNA sequencing. The amplification product and pTYB4 expression vector that has NcoI and SmaI sites on its multiple cloning sites (New England Biolabs, MA) were digested with NcoI and SmaI. The two restriction endonuclease-digested mixtures were combined and ligated with T4 DNA ligase (Promega). The nucleotide sequence of the insert in the construct was verified by DNA sequencing.

[0062] Expression and Purification of VrCRPΔsp

[0063] A nucleic acid sequence (including SEQ ID NO:3) encoding a signal peptide truncated form of VrCRP, denoted as VrCRPΔsp (SEQ ID NO:4 and an N-terminal methionine, and two C-terminal junction residues) was cloned into the pTYB4 expression vector which contains a heterologous bacterial T7 promoter. The resulting construct, pTYB4-VrCRPΔsp, was transferred to *E. coli* BL21(DE3). The *E. coli* transformants were cultured in LB (Luria Bertani) liquid medium containing 100 μg mL^{-1} ampicillin at 37° C. overnight. The overnight culture was diluted to 50-folds with LBA broth and cultured at 37° C. for about 2 h (A_{600}=0.3 to 0.4). Then culture was gently shaken in an ice bath to bring the temperature down to 24° C. and allowed to grow at 24° C. thereafter. IPTG was added to the culture at a final concentration of 0.3 mM and the culture was incubated at 24° C. for 6 h. The culture was then put into an ice bath for 30 min and the *E. coli* cells were harvested by centrifugation at 4° C., 4400×g for 15 min. The expressed VrCRP was purified by intein mediated purification system with an affinity chitin-binding tag according to the method previously described (Chonig et al., 1997, *Gene* 192:271-281). The cells were washed once with distilled water and suspended in a lysis buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 0.1 mM EDTA, 0.1%, Triton X-100, 0.1% TWEEN-20) to obtain a cell suspension (A_{600}=25 to 30). The cell suspension was homogenized with Microfludizer and cell debris were removed by centrifugation at 12,000×g for 10 min. The supernatant was filtered through a 0.45 μm membrane filter. The filtrate containing VrCRP-chitin binding domain (CBD) fusion protein was passed through a chitin affinity column (16×100 mm, bed volume: 30 mL) at a flow rate of 0.4 mL min^{-1}. The column was washed with 15-20-fold bed volume of a washing buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 0.1 mM EDTA, 0.1% Triton X-100, 0.1% TWEEN-20) at a flow rate of 0.9 mL min^{-1}. The VrCRPΔsp-CBD fusion protein bound to the affinity column was cleaved with DTT by introducing 2.5 fold bed volume of a cleavage buffer (20 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.1 mM EDTA, 30 mM DTT) into the column. The column was then saturated with the cleavage buffer and kept at 4° C. for 16 h. VrCRPΔsp was eluted from the column with an elution buffer (20 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.1 mM EDTA). VrCRPΔsp was further purified with FPLC system using Superdex peptide HR 10/30 column (10×300-310 mm, bed volume 24 mL) (Pharmacia) to remove small amounts of contaminating proteins. The column was equilibrated with 20 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.1 mM EDTA, 10 mM β-mercaptoethanol and VrCRPΔsp was eluted with the same buffer at a flow rate of 1.0 mL min^{-1}. The purified VrCRPΔsp was homogeneous as examined by SDS-PAGE.

[0064] Bioassay of VrCRPΔsp Activity

[0065] Activity of VrCRPΔsp against *C. chinensis*, one of the major bruchid pests of mung bean, was studied with artificial mung bean seeds. The artificial seeds were prepared according to the method of Shade et al.((1986) *Bio/Technology* 12:793-796) with modification. The artificial seeds have an average volume and weight of 95 μL and 98.6 mg,

respectively. Each seed is sufficient to hatch six eggs of *C. chinensis*. Thirty artificial seeds were divided into 6 groups for a six-replicate experiment. Ten pairs of *C. chinensis* with equal number of male and female bruchids were introduced into the growth chamber and allowed to lay eggs on the seeds for 24 hours. The number of eggs on the surface of each seed was strictly controlled to six by destroying the extra eggs with a small needle. The seeds were incubated at 25° C. and 60% relative humidity and examined for within-seed development times (WSDT) and percentage emergence at proper time interval. The results were subject to statistic analysis using unpaired student t-test.

[0066] Effect of VrCRPΔsp on the Growth of *Spodoptera frugiperda* Cells

[0067] Fall armyworm (*Spodoptera frugiperda*, Sf 21, Gibco) cells were grown in TNM-FH medium (TNM-FH insect medium, Sigma, T1032) that contained 8% fetal bovine serum (FBS) at 28° C. for 3 days. The cell number was counted under microscope and adjusted to 6×10^5 cells per unit. Appropriate amounts of VrCRPΔsp were added and cultured for 3 days. The number of cells was counted under microscope.

[0068] In Vitro Translation Inhibition Assay

[0069] The reaction mixture contained 0.75 μL potassium acetate (1 M), 0.5 μL BMV (Brome Mosaic Virus) RNA (Promega Co.) (0.5 $\mu g\ \mu L^{31\ 1}$), 2 μL unlabeled amino acid mixture except methionine, 1 mM, 1.9 μL L-[^{35}]methionine (500 μCi, Amersham, AG1094), 12.5 μL wheat germ extract (Promega, L4380), 0.5 μL Rnasin (40 U μL^{-1}) and 10 to 40 [M VrCRPΔsp. The final volume was adjusted to 25 μL, by nuclease-free water. The reaction mixture was incubated at 25° C. for 90 min. Aliquots of the reaction mixture were taken, spotted on to a glass-fiber filter and dried at 45° C. for 3 minutes. The filters were dipped in 10% trichloroacetic acid (TCA) then washed thoroughly with 5% TCA, and then finally washed with 95% ethanol and dried. Radioactivity was measured in a liquid scintillation counter.

[0070] Preparation of Antiserum

[0071] Two New Zealand white rabbits were used for raising antibodies against the purified VrCRPΔsp. The purified VrCRPΔsp was covalently coupled to keyhole limpet hemocyanin (KLH) using the bifunctional reagent glutaraldehyde and mixed with complete adjuvant (TiterMax Antiserum Preparation Gold). The rabbits were injected with the antigen-KLH-adjuvant mixture 3 times with a 4-week interval between injections. The amounts of VrCRPΔsp used for the injections were 150, 100 and 50 μg for the first, second and third injection, respectively. The rabbits were bled from carotid artery one month after the final injection. The blood clot was centrifuged for 10 min at 400×g at 4° C. The clear serum was removed and the complement system was inactivated by incubating at 56° C. for 30 min. The serum was lyophilized and stored at −70° C.

[0072] Western Blot Analysis

[0073] Proteins were resolved with 12.5% SDS-PAGE and trans-blotted to a PVDF (Polyvinylidene fluoride) membrane using capillary transfer (Zeng et al., 1999, *Biotechniques* 26:426-430). The membrane was washed with TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% TWEEN-20) and equilibrated with TBST containing 1% bovine serum albumin. The blot on the membrane was treated with anti-VrCRPΔsp antiserum for 90 min and washed. Then treated with the second antibody (anti-rabbit IgG, AP-linked) for another 90 mml. After washing the membrane was incubated with the coloring reagent 5-bromo-4-chloror-3-indolylphosphate/nitro blue tetrazolium (BCIP/NBT) at 25° C. for 4 min. The reaction was terminated by washing the membrane with distill water for 10 min.

Results

[0074] Isolation of VrCRP cDNA

[0075] Plant lines which exhibit resistance to pests and pathogens, e.g., to bruchids, are a valuable source for the identification of factors which can confer pest and pathogen resistance. Such plants can be bred to another line of the same species, which is sensitive to the pest. Mendelian analysis of the progeny indicates of the number of heterogeneous genes contributing to resistance. Resistant progeny are identified, e.g., by the bruchid resistance assay described below, and repeatedly bred to create a resistant plant line, nearly isogenic to the sensitive plant line. Thus, one can produce two nearly isogenic lines of the mung bean *Vigna radiata*, such as VC1973A and VC6089A. It was known that VC1973A seeds are susceptible to bruchid *Callosobruchus chinensis*, whereas VC6089A seeds are resistant to bruchids. Feeding tests of *C. chinensis* on the seeds of VC1973A and VC6089A revealed that the developing seeds of VC6089A were bruchid resistant around 15 days after flowering (DAF). The two lines of *Vigna radiata* L. VC1973A and VC6089A were obtained from Asian Vegetable Research and Development Center, P.O. Box 42, Shanhua, Tainan 741, Taiwan.

[0076] Subsequently, a variety of methods can be used to identify pest-resistant genes. For example, the resistant gene locus can be genetically mapped to a chromosomal locus. Nucleic acid can be isolated from the locus by a variety of techniques, including the use of markers, walking techniques, genomic libraries, and the polymerase chain reaction.

[0077] Alternatively, differential gene expression analysis techniques can be used to identify the resistant alleles. A tissue is identified which exhibits resistance to the pest. mRNA is isolated from the same tissue from isogenic or nearly isogenic, resistant and sensitive plants, and reverse transcribed. The cDNA population unique to the resistant tissue is identified by subtractive hybridization techniques, which are routine for skilled artisans. After subtraction, cDNAs which are expressed in the resistant, but not the sensitive strain are available for amplification, cloning, and sequencing.

[0078] By using suppression subtractive hybridization a full-length cDNA, named VrCRP, was isolated from the bruchid resistant line *Vignia radiata* L. VC6089A. This cDNA (SEQ ID NO:1) encodes a protein of 73 amino acids containing 8 cysteine residues and a signal peptide of 22 amino acids. The sequence of the encoded protein is recited in SEQ ID NO:2. The VrCRP has a cysteine stabilized α-helix motif(CSH motif) similar to a number of cysteine-rich proteins (Kobayashi et al., 1991, *Biopolymer* 31:1213-1220; Froy et al., 1998, *FASEB J* 12:1793-1796) and shows amino acid sequence homology to fabatin-1 and fabatin-2 isolated from fava bean (*Vicia faba*) (47.6%. identity)

(Zhang et al., 1997, *FEMS Microbiol. Lett.* 149:59-64), α-amylase inhibitor from sorghum (Sorghum bicolor) (42.6% identity) (Bloch et al., 1991, *FEBS Lett.* 279:101-104) and weak homology (less than 40%) to a number of other small cysteine-rich proteins from a variety of organisms. The VrCRP protein also contains a signal sequence. A variant of the VrCRP protein lacking the signal sequence is designated as VrCRPΔsp, and consists of 51 amino acids of VrCRP, commencing with the 23rd residue of VrCRP, and terminating at the final residue of VrCRP. This variant has an additional methionine at its N-terminus, and a proline and glycine at its C-terminus.

[0079] Expression of VrCRP in *E. coli*

[0080] *E. coli* M15 transformed with pQE30 harboring full-length VrCRP failed to express VrCRP. The transformants lysed after the IPTG induction (isopropyl β-D-thiogalactopyranoside). When *E. coli* M15 cells were induced at A_{600}=0.3 to 0.5, cell growth was arrested within one hour. Electron microscopic analysis revealed gross morphological aberrations in the induced cells relative to induced cells lacking VrCRP plasmid. The results indicate that VrCRP is toxic to *E. coli* M15. *E. coli* harboring signal peptide truncated VrCRP (denoted as VrCRPΔsp) grow normally at an early stage similar to control *E. coli* M15 cells, but their growth slowed down after 1 hour, and stopped alter 2 hours. On the other hand, *E. coli* transformants harboring VrCRP failed to grow to an even greater extent.

[0081] Expression was improved by using signal peptide deleted VrCRP in an IMPACT (intein mediated purification with an affinity chitin-binding tag, New England Biolabs, MA) expression system. As described above, VrCRPΔsp is a 54 amino acid protein of which 51 amino acids are derived from VrCRP, while the three remaining (methionine, proline, and glycine) are terminal residues resulting from the cloning vector. The system IMPACT was used to express signal peptide truncated VrCRP. The expressed VrCRPΔsp fusion protein with a molecular mass of 60.9 kDa was bound to a chitin affinity column. The fusion protein was cleaved with DTT (1,4-dithiothreitol) to give rise to a VrCRPΔsp with a molecular mass of 5.9 kDa., and migrates as such on an 4-12% gradient SDS polyacrylamide gel (SDS-PAGE). The eluted VrCRPΔsp was further purified to apparent homogeneity by a Superdex Peptide HR 10/30 column, as determined by electrophoresing 4 μg of the purified protein oil a silver-nitrate stained SDS-PAGE gel. The purified VrCRPΔsp was characterized by its molecular mass (5.9 kDa) and N-terminal amino acid sequencing. The purified VrCRPΔsp showed molecular mass of 5.9 kDa in SDS-PAGE that corresponds to the calculated molecular weight 5,944 kDa. The forty amino acid N-terminal sequence of VrCRPΔsp completely matched to the corresponding nucleotide sequence of VrCRP.

[0082] Bruchid Resistant Activity of VrCRP

[0083] To determine whether VrCRPΔsp plays a role in bruchid resistance or not, artificial seeds containing different amounts (0.01%-0.25%, w/w) of the purified VrCRPΔsp were produced. The seeds were tested for this resistance to *C. chinensis*. Intact mung bean seeds (TN5, VC1973A and VC6089A) and artificial seeds made from the flour of these three mung bean varieties were also tested for comparison. Bruchids were allowed to lay eggs on the intact and artificial seeds. The number of eggs on the surface of each seed was confined to six by destroying the extra eggs. The seeds were incubated at 25° C. and 60% relative humidity. Within-seed development times (WSDT) and percentage emergence are used as criteria for bruchid resistance. The results are shown in Table 1:

TABLE 1

Material Screened	Within seed development time	Percentage Emergence
Intact Seeds		
TN5	26.3 ± 0.6	100 ± 0
VC1973A	25.9 ± 0.9	100 ± 0
VC6089A	—	0 ± 0
Artificial Seeds with from:		
TN5	35.4 ± 1.6	51.3 ± 9.8
VC1973A	36.4 ± 1.6	53.1 ± 5.0
VC6089A	—	0 ± 0
Artificial Seeds with purified protein:		
VC1973A + 0.01% VrCRPΔsp	40.7 ± 2.1	12.5 ± 5.9
VC1973A + 0.09% VrCRPΔsP	48	2.1 ± 5.9
VC1973A + 0.20% VrCRPΔsp	—	0 ± 0
VC1973A + 0.25% VrCRPΔsp	—	0 ± 0
VC1973A + 0.25% BSA	41.0 ± 2.6	52.6 ± 7.9

[0084] In intact seeds of susceptible mung beans, TN5 and VC1973A, 100% of the eggs hatched to produce adult bruchids after approximately 26 days. However, no adults emerged from eggs on intact VC6089A seeds, the bruchid resistant mung bean line.

[0085] Artificial seeds produced from flour from each respective mung bean line showed similar properties. In artificial seeds of susceptible mung beans, TN5 and VC1973A, greater than 50% of the eggs hatched to produced adult bruchids after approximately 35 days. Again, no adults emerged from artificial eggs made from intact VC6089A seeds, the bruchid resistant mung bean line.

[0086] Artificial seeds were used to test the efficacy of purified recombinant VrCRPΔsp. Artificial seed were produced with flour from the susceptible mung bean VC1973A combined with 0.01%, 0.09%, 0.20%, and 0.25% VrCRPΔsp. The seeds were compared to artificial seeds with 0.25% bovine serum albumin (BSA). The VrCRPΔsp at 0.01% concentration in the artificial seeds reduced the number of emerging bruchids to 12.5% compared to greater than 50% in the BSA control. Moreover, artificial seeds containing 0.2% VrCRPΔsp completely arrested bruchid development, as no adults emerged from such seeds. Artificial seeds with VrCRPΔsp at this concentration killed bruchid larvae at their first instar stage. Thus, a significant dosage response was observed between the VrCRPΔsp concentrations of 0.01%-0.2%. Thus purified recombinant VrCRPΔsp polypeptide is a potent antagonist of bruchid development and/or viability.

[0087] The performance of *C. chinensis* larvae reared on the artificial seeds containing various amounts (0.01%-0.25%) of VrCRPΔsp was also investigated. Six artificial seeds containing six eggs each were prepared for each dose. Two capsules containing six seeds each were opened after 21, 24, and 27 days respectively. The larvae were counted and weighted. The results were significant, e.g., P<0.035 for

control compared to 0.01% VrCRPΔsp at 24 days, and P<0.001 for 0.01% VrCRPΔsp compared to 0.2% at 21 clays. Thus, artificial seeds containing 0.01% VrCRPΔsp significantly retarded larva development. Complete growth arrest was observed with seeds containing either 0.2% or 0.25% VrCRPΔsp (Table 2). These results provide additional evidence to support the toxicity of VrCRPΔsp to *C. chinensis*.

TABLE 2

Performance of *C. chinensis* larvae on artificial seeds containing VrCRPΔsp.

	mean weight of larvae (mg)			
	Control (0%)	0.01% VrCRPΔsp	0.2% VrCRPΔsp	0.25% VrCRPΔsp
Day 21	5.2	3.1	0	0
Day 24	6.6	4.6	0	0
Day 27	8	5.2	0	0

[0088] VrCRPΔsp Inhibits Protein Synthesis

[0089] The purified recombinant VrCRPΔsp polypeptide was combined with in vitro translation extracts to determine the effect of the polypeptide on translation. A 40 μM concentration of VrCRPΔsp protein completely inhibited the incorporation of amino acids including p355] methionine into acid insoluble fraction. A significant dosage response was observed between 10-40 μM of VrCRPΔsp. With 10 LM VrCRPΔsp, a greater than 60% reduction in acid insoluble counts was observed, with 40 μM, a greater than 80% reduction. The results indicated that VrCRPΔsp is a strong inhibitor of protein biosynthesis.

[0090] Effect of VrCRPΔsp on Growth of *Spodoptera frugiperda* Cells

[0091] VrCRPΔsp was added to the culture medium of an insect cell culture, a culture with cells of the fall armyworm (*Spodoptera frugiperda, Sf* 21). After VrCRPΔsp addition, cells were incubated at 28° C. and counted after three days. Purified VrCRPΔsp at 3.42 μM completely arrested the growth of Sf 21 cells. Rupture of Sf 21 cells under the conditions was observed by phase-contrast microscopy. The concentration of VrCRPΔsp that caused LC_{50} (50% lethality concentration) was 1.7 μM.

[0092] Antifungal Activity of VrCRPΔsp

[0093] Sterile filter discs were prepared containing 0, 1, 2, 3, 6, 10, 20, and 40 μg of purified VrCRPΔsp in 20 mM TrisHCl pH 8.0, 50 mM NaCl, 0.1 mM EDTA. The filter discs were placed on agar plates which had been top spread with the fungus *Rhizoctonia solani*. The plates were incubated at 28° C. for 36 to 49 hours. The radius of the zone of inhibition was measured surrounding each disc. A zone of inhibition greater than 2 mm was observed for filter discs containing 10, 20, and 40 μg of purified VrCRPΔsp, indicating that at these concentrations, VrCRPΔsp has antifungal activity.

[0094] Additional Compositions

[0095] It was described previously that a single dominant bruchid resistance gene (Br) in the wild mung bean accession TC1966 has been transferred a susceptible cultivar and a resistant isogenic ($BC_{20}F_4$) line was developed by selection for bruchid resistance against the azuki bean weevil. Two novel cyclopeptide alkaloids, denoted vignatic acid A and B were identified in the resistant isogenic line. The addition of vignatic acid A to pellets of mung bean flour at concentrations more than 1% resulted in the complete elimination of the bruchids, but vignatic acid B had no observable effect on bruchids (Sugawara et al, 1996, *J. Agric. Food Chem.* 44:3360-3364; Kaga et al., 1998, *Mol. Gen. Genet.* 258:378-384). However, the insecticidal activity of vignatic acid A alone was unable to explain the resistance of the resistant isogenic line to azuki bean weevil.

[0096] Other embodiments are within the following claims.

SEQUENCE LISTING

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<212> TYPE: DNA
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<220> FEATURE:
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<222> LOCATION: (21)...(239)

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ctc ctt ctc gtc tta gcc tct gat gtg gcc gta gag aga gga gag gct      101
Leu Leu Leu Val Leu Ala Ser Asp Val Ala Val Glu Arg Gly Glu Ala
            15                  20                  25

aga act tgt atg ata aag aaa gaa ggg tgg gga aaa tgc tta att gac      149
Arg Thr Cys Met Ile Lys Lys Glu Gly Trp Gly Lys Cys Leu Ile Asp
        30                  35                  40
```

-continued

```
acc acc tgt gca cat tcg tgc aag aac cgc ggt tac ata ggt gga gat      197
Thr Thr Cys Ala His Ser Cys Lys Asn Arg Gly Tyr Ile Gly Gly Asp
     45                  50                  55

tgc aaa ggc atg acg cgc acc tgc tat tgc ctc gtc aac tgt              239
Cys Lys Gly Met Thr Arg Thr Cys Tyr Cys Leu Val Asn Cys
 60                  65                  70

tgaacccttt tcgaatatca tatcatctta tcacaaataa atatagcagc atcactgcta    299

ctagtaccgc cctccgcacc acgccct                                       326


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<213> ORGANISM: Vigna radiata

<400> SEQUENCE: 2

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 1               5                  10                  15

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Lys Lys Glu Gly Trp Gly Lys Cys Leu Ile Asp Thr Thr Cys Ala His
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Ser Cys Lys Asn Arg Gly Tyr Ile Gly Gly Asp Cys Lys Gly Met Thr
    50                  55                  60

Arg Thr Cys Tyr Cys Leu Val Asn Cys
65                  70


<210> SEQ ID NO 3
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<212> TYPE: DNA
<213> ORGANISM: Vigna radiata
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 1               5                  10                  15

aaa tgc tta att gac acc acc tgt gca cat tcg tgc aag aac cgc ggt       96
Lys Cys Leu Ile Asp Thr Thr Cys Ala His Ser Cys Lys Asn Arg Gly
            20                  25                  30

tac ata ggt gga gat tgc aaa ggc atg acg cgc acc tgc tat tgc ctc      144
Tyr Ile Gly Gly Asp Cys Lys Gly Met Thr Arg Thr Cys Tyr Cys Leu
        35                  40                  45

gtc aac tgt tga                                                     156
Val Asn Cys
    50


<210> SEQ ID NO 4
<211> LENGTH: 51
<212> TYPE: PRT
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 1               5                  10                  15

Lys Cys Leu Ile Asp Thr Thr Cys Ala His Ser Cys Lys Asn Arg Gly
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Val Asn Cys
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<212> TYPE: DNA
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<220> FEATURE:
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tcccccggga cagttgacga ggcaata 27

What is claimed is:

1. A purified polypeptide comprising an amino acid sequence which is at least 60% identical to the amino acid sequence of SEQ ID NO:4, wherein the polypeptide has insecticidal or fungicidal activity.

2. The purified polypeptide of claim 1 wherein the polypeptide inhibits translation of a messenger RNA in a wheat germ extract.

3. A composition comprising the polypeptide of claim 1 present in an amount of 0.01% to 10% by weight of composition.

4. The composition of claim 3 wherein the polypeptide is present in an amount of 0.05% to 5% by weight of composition.

5. The polypeptide of claim 1 wherein the amino acid sequence is at least 80% identical to the amino acid sequence of SEQ ID NO:4.

6. The purified polypeptide of claim 5 wherein the polypeptide inhibits translation of a messenger RNA in a wheat germ extract.

7. The polypeptide of claim 5 wherein the amino acid sequence is at least 90% identical to the amino acid sequence of SEQ ID NO:2.

8. The purified polypeptide of claim 7 wherein the polypeptide inhibits translation of a messenger RNA in a wheat germ extract.

9. A purified polypeptide comprising the amino acid sequence of SEQ ID NO:4.

10. A purified polypeptide comprising the amino acid sequence of SEQ ID NO:4, with up to 18 conservative amino acid substitutions, wherein the polypeptide has insecticidal or fungicidal activity.

11. A purified polypeptide encoded by a nucleic acid that hybridizes under high stringency conditions to a probe the sequence of which consists of SEQ ID NO: 1, wherein the polypeptide has insecticidal or fungicidal activity.

12. An isolated nucleic acid encoding the polypeptide of claim 1.

13. The isolated nucleic acid of claim 12 further comprising an operably linked heterologous promoter.

14. An isolated nucleic acid encoding the polypeptide of claim 5.

15. The isolated nucleic acid of claim 14 further comprising an operably linked heterologous promoter.

16. An isolated nucleic acid encoding the polypeptide of claim 9.

17. The isolated nucleic acid of claim 16 further comprising an operably linked heterologous promoter.

18. An isolated nucleic acid comprising a strand that hybridizes under high stringency conditions to a single stranded probe, the sequence of which consists of SEQ ID NO:3 or the complement of SEQ ID NO:3.

19. The isolated nucleic acid of claim 18, wherein the nucleic acid encodes a polypeptide that has insecticidal or fungicidal activity.

20. An isolated nucleic acid comprising a strand that hybridizes under high stringency conditions to a single stranded probe, the sequence of which consists of SEQ ID NO: 1 or the complement of SEQ ID NO: 1, wherein the nucleic acid encodes a polypeptide that has insecticidal or fungicidal activity.

* * * * *

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L5: Entry 26 of 28 File: DWPI Jan 8, 2004

DERWENT-ACC-NO: 2004-081727
DERWENT-WEEK: 200408

TITLE: New mung bean thionin polypeptide and nucleic acid, useful in generating transgenic plants having insecticidal activity against bruchids, such as Callosobruchus chinensis, or fungicidal activity against Rhizoctonia solani

Basic Abstract Text (6):
ACTIVITY - Insecticide; Fungicide; Pesticide. Sterile filter discs were prepared containing 0, 1, 2, 3, 6, 10, 20 and 40 micro g of purified VrCRP Delta sp (a signal peptide truncated form of mung bean thionin polypeptide) in 20 mM Tris-hydrochloride, 50 mM sodium chloride and 0.1 mM ethylene diamine tetraacetate (EDTA). The filter discs were placed on agar plates which had been top spread with the fungus Rhizoctonia solani. The plates were incubated at 28 deg. C for 36-49 hours. A zone of inhibition greater than 2 mm was observed for filter discs containing 10, 20 and 40 micro g of purified VrCRP Delta sp, indicating that at these concentrations, VrCRP Delta sp has anti-fungal activity.

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(54) BACTERICIDAL COMPOSITION CONTAINING PEPTIDE AND CHELATING AGENT

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(52) U.S. Cl. 424/94.63; 514/564

(57) ABSTRACT

A bactericidal composition is provided, which comprises as effective ingredients (a) at least one substance selected from the group consisting of ethylenediaminetetraacetic acid and metal salts thereof and (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin. The bactericidal composition, for example, has the effect of sterilizing food poisoning bacteria at a low concentration and is highly safe.

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(54) Title: HIGH THREONINE DERIVATIVES OF α-HORDOTHIONIN

(57) Abstract

Derivatives of α-hordothionin made by position-specific substitution with threonine residues provide threonine in plants.

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HIGH THREONINE DERIVATIVES OF α-HORDOTHIONIN

TECHNICAL FIELD

This invention relates to the improvement of feed formulations. Specifically, this invention relates to derivatives of α-hordothionin which provide higher percentages of threonine in plants.

BACKGROUND OF THE INVENTION

Feed formulations are required to provide animals essential nutrients critical to growth. However, crop plants are generally rendered food sources of poor nutritional quality because they contain low proportions of several amino acids which are essential for, but cannot be synthesized by, animals.

For many years researchers have attempted to improve the balance of essential amino acids in the proteins of important crops through breeding programs. As more becomes known about storage proteins and the expression of the genes which encode these proteins, and as transformation systems are developed for a greater variety of plants, molecular approaches for improving the nutritional quality of seed proteins can provide alternatives to the more conventional approaches. Thus, specific amino acid levels can be enhanced in a given crop via biotechnology.

One alternative method is to express a heterologous protein of favorable amino acid composition at levels sufficient to obviate food or feed supplementation. For example, a number of seed proteins rich in sulfur amino acids have been identified. A key to good expression of such proteins involves efficient expression cassettes with seed specific promoters. Not only must the gene-controlling regions direct the synthesis of high levels of mRNA, the mRNA must be translated into stable protein.

Among the essential amino acids needed for animal nutrition, often missing from crop plants, are methionine,

threonine and lysine. Attempts to increase the levels of these free amino acids by breeding, mutant selection and/or changing the composition of the storage proteins accumulated in crop plants has met with minimal success. Usually, the expression of the transgenic storage protein was too low. The phaseolin-promoted Brazil nut 2S expression cassette is an example of an effective chimeric seed-specific gene. However, even though Brazil nut protein increases the amount of total methionine and bound methionine, thereby improving nutritional value, there appears to be a threshold limitation as to the total amount of methionine that is accumulated in the seeds. The seeds remain insufficient as sources of methionine.

An alternative to the enhancement of specific amino acid levels by altering the levels of proteins containing the desired amino acid is modification of amino acid biosynthesis. Recombinant DNA and gene transfer technologies have been applied to alter enzyme activity catalyzing key steps in the amino acid biosynthetic pathway. Glassman, U.S. Patent No. 5,258,300; Galili, et al., European Patent Application No. 485970; (1992); incorporated herein in its entirety by reference. However, modification of the amino acid levels in seeds is not always correlated with changes in the level of proteins that incorporate those amino acids. Burrow, et al., Mol. Gen. Genet.; Vol. 241; pp. 431-439; (1993); incorporated herein in its entirety by reference. Although significant increases in free lysine levels in leaves have been obtained by selection for DHDPS mutants or by expressing the E. coli DHDPS in plants, it remains to be shown that these alterations can increase bound target amino acids, which represent some 90% or more of total amino acids. Thus, there is minimal impact on the nutritional value of seeds.

Based on the foregoing, there exists a need for methods of increasing the levels of the essential amino acids, threonine, methionine and lysine in seeds of plants.

It is therefore an object of the present invention to provide methods for genetically modifying plants to increase the levels of the essential amino acid threonine in the plants.

It is a further object of the present invention to provide seeds for food and/or feed with higher levels of the essential amino acid threonine than wild species of the same seeds.

DISCLOSURE OF THE INVENTION

It has now been determined that one class of compounds, the α-hordothionins, can be modified to enhance their content of threonine. α-hordothionin is a 45-amino acid protein which has been well characterized. It can be isolated from seeds of barley (Hordeum vulgare). The molecule is stabilized by four disulfide bonds resulting from eight cysteine residues. The amino acid sequence is as provided in SEQUENCE I.D. No.1. In its native form, it is especially rich in arginine and lysine residues, containing 5 residues (10%) of each. However, it contains only 3 residues (7%) of the essential amino acid threonine.

The protein has been synthesized and the three-dimensional structure determined by computer modeling. The modeling of the protein predicts that the ten charged residues (arginine at positions 5,10,17,19 and 30, and lysine at positions 1,23,32,38 and 45) all occur on the surface of the molecule. The side chains of the polar amino acids (asparagine at position 11, glutamine at position 22 and threonine at position 41) also occur on the surface of the molecule. Furthermore, the hydrophobic amino acids (such as the side chains of leucine at positions 8,15 24 and 33 and valine at position 18) are also solvent-accessible.

Three-dimensional modeling of the protein indicates that the arginine residue at position 10 is critical to retention of the appropriate 3-dimensional structure and

possible folding through hydrogen bond interactions with the C-terminal residue of the protein. A threonine substitution at that point would disrupt the hydrogen bonding involving arginine at position 10, serine at position 2 and lysine at position 45, leading to destabilization of the structure. The synthetic peptide having this substitution could not be made to fold correctly, which supported this analysis. Conservation of the arginine residue at position 10 provided a protein which folded correctly.

Since threonine is a polar amino acid, the surface polar amino acid residues, asparagine at position 11 and glutamine at position 22, were substituted; and the charged amino acids, lysine at positions 1,23,32 and 38 and arginine at positions 5,17,19, and 30, were substituted with threonine. The resulting compound has the sequence indicated in SEQUENCE I.D. No. 2. The molecule can be synthesized by solid phase peptide synthesis and folds into a stable structure. It has 13 threonine residues (29%).

While SEQUENCE I.D. No. 2 is illustrative of the present invention, it is not intended to be a limitation. Threonine substitutions can also be performed at positions containing charged amino acids. Only arginine at position 10 and lysine at position 45 are critical for maintaining the structure of the protein. One can also substitute at the sites having hydrophobic amino acids. These include positions 8,15,18 and 24. The resulting compound has the sequence indicated in SEQUENCE I.D. NO. 3.

Synthesis of the compounds is performed according to methods of peptide synthesis which are well known in the art and thus constitute no part of this invention. In vitro, the compounds have been synthesized on an applied biosystems model 431a peptide synthesizer using fastmoc™ chemistry involving hbtu [2-(1h-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, as published by Rao, et al., Int. J. Pep. Prot. Res.; Vol. 40; pp. 508-515; (1992); incorporated herein in its entirety by reference.

Peptides were cleaved following standard protocols and purified by reverse phase chromatography using standard methods. The amino acid sequence of each peptide was confirmed by automated edman degradation on an applied biosystems 477a protein sequencer/120a pth analyzer. More preferably, however, the compounds of this invention are synthesized in vivo by bacterial or plant cells which have been transformed by insertion of an expression cassette containing a synthetic gene which when transcribed and translated yields the desired compound. Such empty expression cassettes, providing appropriate regulatory sequences for plant or bacterial expression of the desired sequence, are also well-known, and the nucleotide sequence for the synthetic gene, either RNA or DNA, can readily be derived from the amino acid sequence for the protein using standard reference texts. Preferably, such synthetic genes will employ plant-preferred codons to enhance expression of the desired protein.

Industrial Applicability

The following description further exemplifies the compositions of this invention and the methods of making and using them. However, it will be understood that other methods, known by those of ordinary skill in the art to be equivalent, can also be employed.

Plants

The genes which code for these compounds can be inserted into an appropriate expression cassette and introduced into cells of a plant species. Thus, an especially preferred embodiment of this method involves inserting into the genome of the plant a DNA sequence coding for a compound of this invention in proper reading frame, together with transcription initiator and promoter sequences active in the plant. Transcription and translation of the DNA sequence under control of the regulatory sequences causes expression of the protein sequence at levels which provide an elevated amount of the protein in the tissues of the plant.

Preferred plants that are to be transformed according to the methods of this invention are cereal crops, including maize, rye, barley, wheat, sorghum, oats, millet, rice, triticale, sunflower, alfalfa, rapeseed and soybean.

Synthetic DNA sequences can then be prepared which code for the appropriate sequence of amino acids, and this synthetic DNA sequence can be inserted into an appropriate plant expression cassette.

Likewise, numerous plant expression cassettes and vectors are well known in the art. By the term "expression cassette" is meant a complete set of control sequences including initiation, promoter and termination sequences which function in a plant cell when they flank a structural gene in the proper reading frame. Expression cassettes frequently and preferably contain an assortment of restriction sites suitable for cleavage and insertion of any desired structural gene. It is important that the cloned gene have a start codon in the correct reading frame for the structural sequence.

In addition, the plant expression cassette preferably includes a strong constitutive promoter sequence at one end to cause the gene to be transcribed at a high frequency, and a poly-a recognition sequence at the other end for proper processing and transport of the messenger RNA. An example of such a preferred (empty) expression cassette into which the cDNA of the present invention can be inserted is the pPHI414 plasmid developed by Beach, et al., of Pioneer Hi-Bred International, Inc., Johnston, IA, as disclosed in U.S. patent application No. 07/785,648, (1991); incorporated herein in its entirety by reference. Highly preferred plant expression cassettes will be designed to include one or more selectable marker genes, such as kanamycin resistance or herbicide tolerance genes.

By the term "vector" herein is meant a DNA sequence which is able to replicate and express a foreign gene in a host cell. Typically, the vector has one or more endonuclease recognition sites which may be cut in a

predictable fashion by use of the appropriate enzyme such vectors are preferably constructed to include additional structural gene sequences imparting antibiotic or herbicide resistance, which then serve as markers to identify and separate transformed cells. Preferred markers/selection agents include kanamycin, chlorosulfuron, phosphonothricin, hygromycin and methotrexate. A cell in which the foreign genetic material in a vector is functionally expressed has been "transformed" by the vector and is referred to as a "transformant."

A particularly preferred vector is a plasmid, by which is meant a circular double-stranded DNA molecule which is not a part of the chromosomes of the cell.

As mentioned above, both genomic and cDNA encoding the gene of interest may be used in this invention. The vector of interest may also be constructed partially from a cDNA clone and partially from a genomic clone. When the gene of interest has been isolated, genetic constructs are made which contain the necessary regulatory sequences to provide for efficient expression of the gene in the host cell. According to this invention, the genetic construct will contain (a) a first genetic sequence coding for the protein or trait of interest and (b) one or more regulatory sequences operably linked on either side of the structural gene of interest. Typically, the regulatory sequences will be selected from the group comprising of promoters and terminators. The regulatory sequences may be from autologous or heterologous sources.

Promoters that may be used in the genetic sequence include NOS, OCS and CaMV promoters.

An efficient plant promoter that may be used is an overproducing plant promoter. Overproducing plant promoters that may be used in this invention include the promoter of the cholorophyll α-β binding protein and the promoter of the small sub-unit (ss) of the ribulose-1,5-biphosphate carboxylase from soybean. See e.g. Berry-Lowe, et al., J. Molecular and App. Gen.; Vol. 1; pp. 483-498;

(1982); incorporated herein by reference. These two promoters are known to be light-induced, in eukaryotic plant cells. See e.g., An Agricultural Perspective, A. Cashmore, Pelham, New York; pp. 29-38; (1983); G. Coruzzi, et al., J. Biol. Chem.; Vol. 258; p. 1399; (1983); and P. Dunsmuir, et al., J. Molecular and App. Gen.; Vol. 2; p. 285; (1983); all incorporated herein by reference.

The expression cassette comprising the structural gene for the protein of this invention operably linked to the desired control sequences can be ligated into a suitable cloning vector. In general, plasmid or viral (bacteriophage) vectors containing replication and control sequences derived from species compatible with the host cell are used. The cloning vector will typically carry a replication origin, as well as specific genes that are capable of providing phenotypic selection markers in transformed host cells. Typically, genes conferring resistance to antibiotics or selected herbicides are used. After the genetic material is introduced into the target cells, successfully transformed cells and/or colonies of cells can be isolated by selection on the basis of these markers.

Typically, an intermediate host cell will be used in the practice of this invention to increase the copy number of the cloning vector. With an increased copy number, the vector containing the gene of interest can be isolated in significant quantities for introduction into the desired plant cells. Host cells that can be used in the practice of this invention include prokaryotes, including bacterial hosts such as E. coli, S. typhimurium, and Serratia marcescens. Eukaryotic hosts such as yeast or filamentous fungi may also be used in this invention. Since these hosts are also microorganisms, it will be essential to ensure that plant promoters which do not cause expression of the protein in bacteria are used in the vector.

The isolated cloning vector will then be introduced into the plant cell using any convenient technique,

including electroporation (in protoplasts), retroviruses, bombardment, and microinjection into cells from monocotyledonous or dicotyledonous plants in cell or tissue culture to provide transformed plant cells containing as foreign DNA at least one copy of the DNA sequence of the plant expression cassette. Preferably, the monocotyledonous species will be selected from maize, sorghum, wheat or rice, and the dicotyledonous species will be selected from soybean, alfalfa, rapeseed, sunflower or tomato. Using known techniques, protoplasts can be regenerated and cell or tissue culture can be regenerated to form whole fertile plants which carry and express the gene for a protein according to this invention. Accordingly, a highly preferred embodiment of the present invention is a transformed maize plant, the cells of which contain as foreign DNA at least one copy of the DNA sequence of an expression cassette of this invention.

It will also be appreciated by those of ordinary skill that the plant vectors provided herein can be incorporated into agrobacterium tumefaciens, which can then be used to transfer the vector into susceptible plant cells, primarily from dicotyledonous species. Thus, this invention provides a method for increasing threonine levels in agrobacterium tumefaciens-susceptible dicotyledonous plants in which the expression cassette is introduced into the cells by infecting the cells with agrobacterium tumefaciens, a plasmid of which has been modified to include a plant expression cassette of this invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Pioneer Hi-Bred International, Inc.

(ii) TITLE OF INVENTION: High Threonine Derivatives of Alpha-Hordothionin

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE:Pioneer Hi-Bred International, Inc.
(B) STREET: 700 Capital Square, 400 Locust Street
(C) CITY: Des Moines
(D) STATE: Iowa
(E) COUNTRY: United States of America
(F) ZIP: 50309

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Patent In Release #1.0, Version#1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Simon, Soma G.
(B) REGISTRATION NUMBER: 37,444
(C) REFERENCE/DOCKET NUMBER: 354-PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 515-248-4896
(B) TELEFAX: 515-248-4844

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Lys Ser Cys Cys Arg Ser Thr Leu Gly Arg Asn Cys Tyr Asn Leu Cys
1               5                  10                  15

Arg Val Arg Gly Ala Gln Lys Leu Cys Ala Gly Val Cys Arg Cys Lys
```

20 25 30

Leu Thr Ser Ser Gly Lys Cys Pro Thr Gly Phe Pro Lys
35 40 45

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Thr Ser Cys Cys Thr Ser Thr Leu Gly Arg Thr Cys Tyr Asn Leu Cys
1 5 10 15

Thr Val Thr Gly Ala Thr Thr Leu Cys Ala Gly Val Cys Thr Cys Thr
20 25 30

Leu Thr Ser Ser Gly Thr Cys Pro Thr Gly Phe Pro Lys
35 40 45

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Thr Ser Cys Cys Thr Ser Thr Thr Gly Lys Thr Cys Tyr Asn Thr Cys
1 5 10 15

Thr Thr Thr Arg Ala Thr Thr Thr Cys Ala Gly Val Cys Thr Cys Thr
20 25 30

Leu Thr Ser Ser Gly Thr Cys Pro Thr Gly Phe Pro Lys
35 40 45

WHAT IS CLAIMED IS:

1.

A protein having the sequence of SEQUENCE I.D. No. 3 wherein the amino acid residues at one or more of positions 1,5,7,8,11,15,17,18,19,22,23,24,30,32,34,38 and 41 are threonine, and the remainder of the residues at those positions are the residues at the corresponding positions in SEQUENCE I.D. No. 1.

2.

The protein of Claim 1 wherein one or more of the amino acid residues at 1,5,7,11,17,19,22,23,30,32,34,38 and 41 are threonine.

3.

The protein of Claim 2 wherein at least 5 of the amino acid residues at positions 1,5,7,11,17,19,22,23,30,32,34,38 and 41 are threonine.

4.

The protein of Claim 3 wherein at least 7 of the amino acid residues at positions 1,5,7,11,17,19,22,23,30,32,34,38 and 41 are threonine.

5.

A nucleotide sequence which codes for a protein having the sequence of SEQUENCE I.D. No. 3 wherein the amino acid residues at one or more of positions 1,5,7,8,11,15,17,18,19,22,23,24,30, 32,34,38 and 41 are threonine, and the remainder of the residues at those positions are the residues at the corresponding positions in SEQUENCE I.D. No. 1.

6.

An RNA sequence which codes for a protein having the sequence of SEQUENCE I.D. No. 3 wherein the amino acid residues at one or more of positions 1,5,7,8,11,15,17,18,19,22,23,24,30, 32,34,38 and 41 are threonine, and the remainder of the residues at those positions are the residues at the corresponding positions in SEQUENCE I.D. No. 1.

7.

A DNA sequence which codes for a protein having the sequence of SEQUENCE I.D. No. 3 wherein the amino acid residues at one or more of positions 1,5,7,8,11,15,17,18,19,22,23,24,30,32,34,38 and 41 are threonine, and the remainder of the residues at those positions are the residues at the corresponding positions in SEQUENCE I.D. No. 1.

8.

An expression cassette containing the DNA sequence of Claim 7 operably linked to plant regulatory sequences which cause the expression of the DNA sequence in plant cells.

9.

A bacterial transformation vector comprising an expression cassette according to Claim 8, operably linked to bacterial expression regulatory sequences which cause replication of the expression cassette in bacterial cells.

10.

Bacterial cells containing as a foreign plasmid at least one copy of a bacterial transformation vector according to Claim 9.

11.

Transformed plant cells containing at least one copy of the expression cassette according to Claim 8.

12.

The transformed plant cells of Claim 11, wherein the cells are of a monocotyledonous species.

13.

The transformed plant cells of Claim 12, wherein the cells are selected from the group consisting of maize, sorghum, wheat and rice cells.

14.

The transformed cells of Claim 11, wherein the cells are of a dicotyledonous species.

15.

The transformed cells of Claim 14, wherein cells are selected from the group consisting of soybean, alfalfa, rapeseed, sunflower, tobacco and tomato cells.

16.

A maize cell or tissue culture comprising cells according to Claim 13.

17.

A method for enhancing the threonine content of a plant cell or seed comprising the step of expressing a protein according to Claim 1 in the cell or seed.

18.

The method of Claim 17 wherein the plant is a dicotyledonous plant.

19.

The method of Claim 17 wherein the plant is a monocotyledonous plant.

20.

The method of Claim 19 wherein the threonine content of the plant seed is enhanced.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 96/08219

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/82 C07K14/415 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO,A,94 16078 (PIONEER HI BRED INT) 21 July 1994 see the whole document ---	1,2,5-16 1,2,5-20
Y	THE PLANT JOURNAL, vol. 3 , no. 5, 1993, pages 721-727, XP002015695 KARCHI, H., ET AL.: "Seed-specific expression of a bacterial desensitized aspartate kinase increases the production of seed threonine and methionine in transgenic tobacco" see page 726, left-hand column, line 33 - line 37 --- -/--	1,2,5-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 1996	28. 10. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Maddox, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROTEIN ENGINEERING, vol. 7, no. 12, 1 December 1994, pages 1485-1493, XP000482988 RAO A G ET AL: "STRUCTURE-FUNCTION VALIDATION OF HIGH LYSINE ANALOGS OF -HORDOTHIONIN DESIGNED BY PROTEIN MODELING"	1,2,5-7
A	see the whole document	3,4,8-20
A	PLANT MOLECULAR BIOLOGY, vol. 24, 1994, pages 83-96, XP002015812 FLORACK, D.E.A., ET AL.: "Expression of biologically active hordothionins in tobacco. Effects of pre- and pro-sequences at the amino and carboxyl termini of the hordothionin precursor on mature protein expression and sorting" see the whole document	8-16
A	WO,A,94 10315 (PIONEER HI BRED INT) 11 May 1994 see page 14, line 1 - line 14; claim 1	1-20
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, March 1994, WASHINGTON US, pages 2577-2581, XP002015697 KARCHI, H., ET AL.: "Lysine synthesis and catabolism are coordinately regulated during tobacco seed development" see page 2581, left-hand column, last line - right-hand column	1-20
A	WO,A,89 04371 (UNIV LOUISIANA STATE) 18 May 1989 see page 18, line 25 - page 24, line 10	1-20
A	EP,A,0 318 341 (PLANT GENETIC SYSTEMS NV) 31 May 1989 see page 5, line 60 - page 6, line 57	1-20
A	WO,A,93 19190 (DU PONT) 30 September 1993 see the whole document	1-20
A	WO,A,93 03160 (DU PONT) 18 February 1993 see the whole document	1-20

3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/08219

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